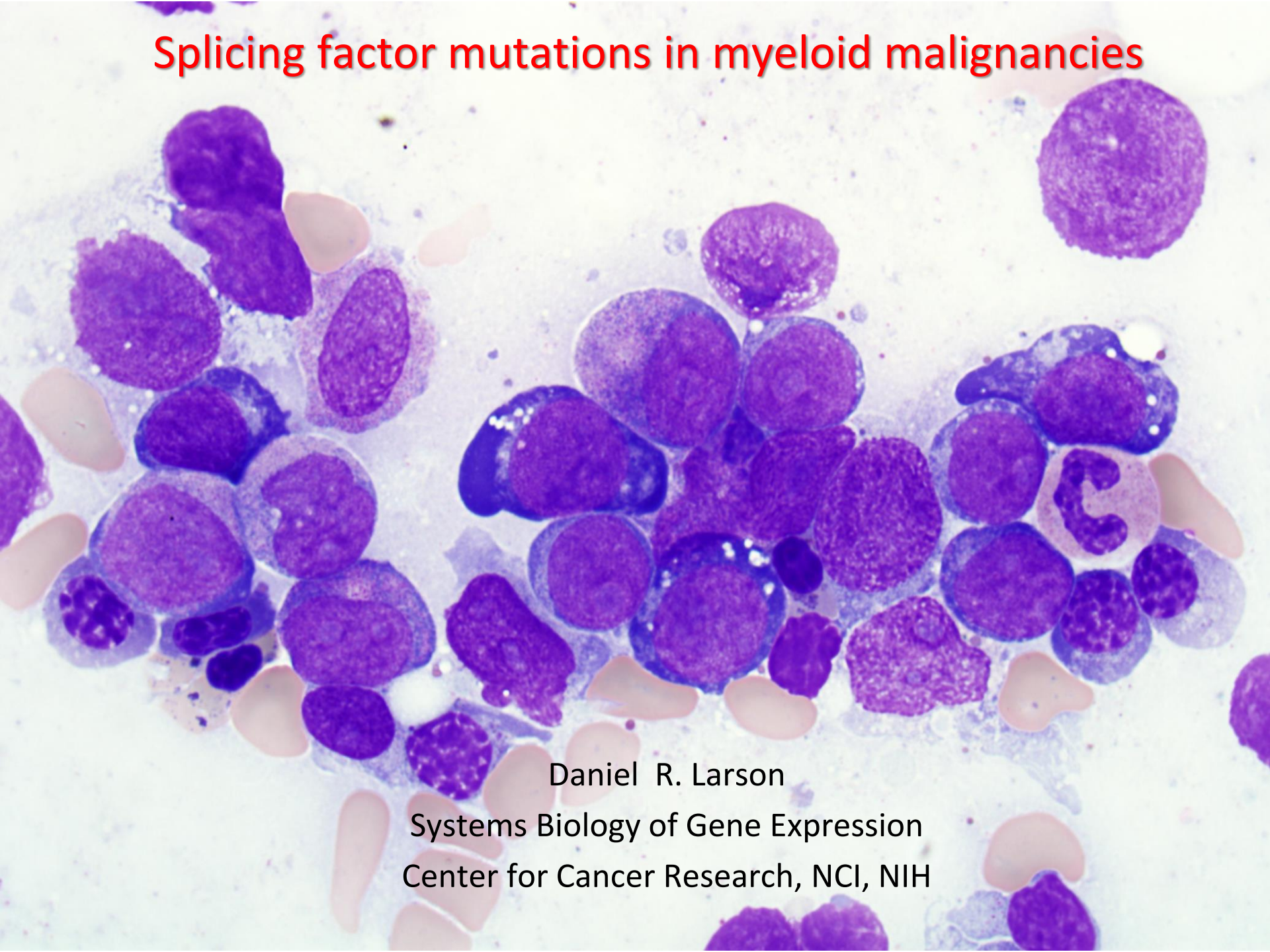
Splicing factor mutations in myeloid malignancies



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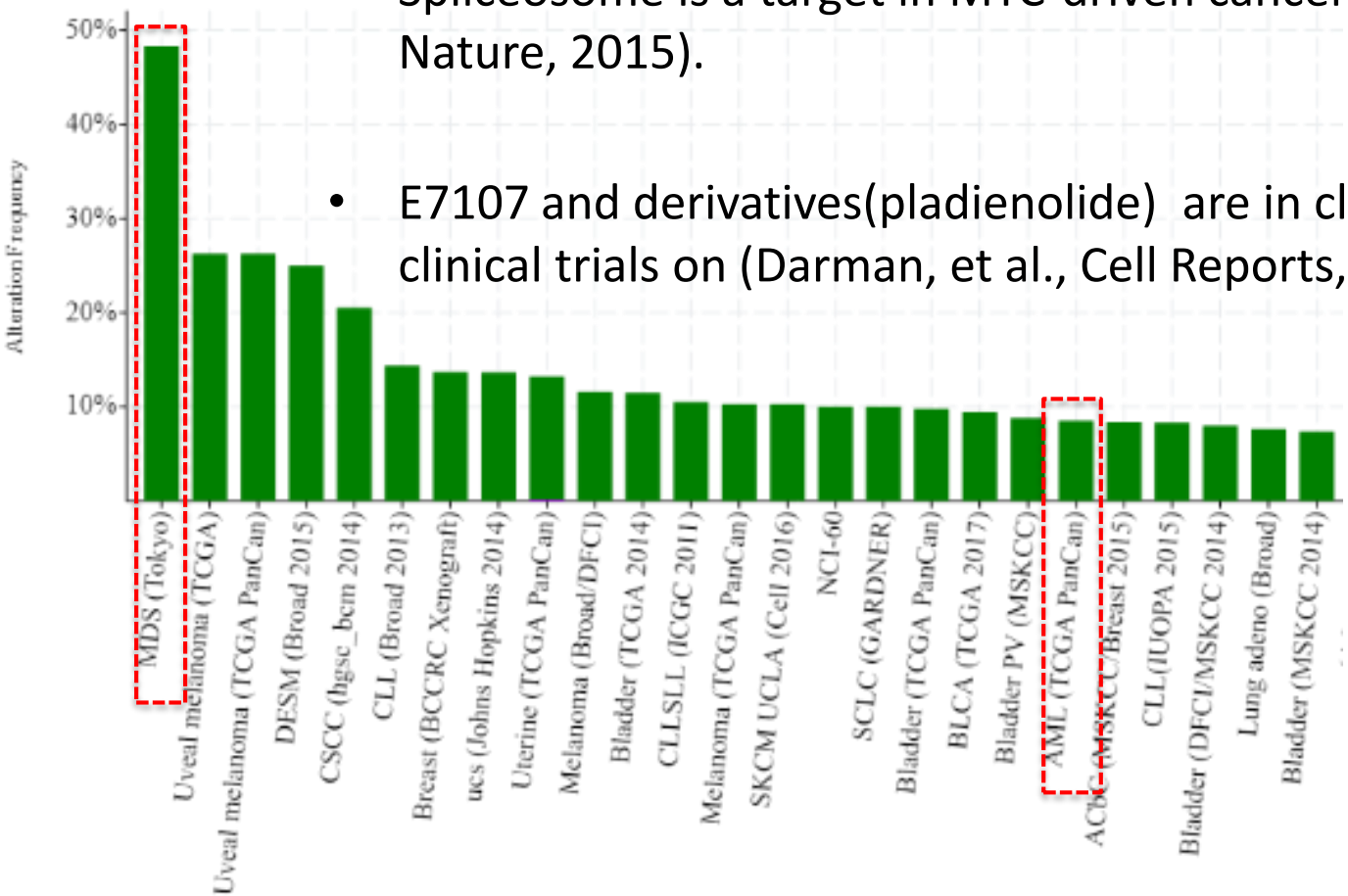
Spliceosome mutations have emerged in almost every tumor type

- Splicing factor mutations occur in ~ 50% of myelodysplasia (Dolatshad et al., Leukemia, 2015).
- Spliceosome is a target in MYC-driven cancers (Hsu et al., Nature, 2015).
- E7107 and derivatives(pladienolide) are in clinical and pre-clinical trials on (Darman, et al., Cell Reports, 2015).



SF3B1, SRSF2, U2AF1, ZRSR2 mutations, The Cancer Genome Atlas (June, 2018)

Disease progression in myeloid malignancies

nature medicine LETTERS
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Corrected: Publisher Correction

Myelodysplastic syndrome progression to acute myeloid leukemia at the stem cell level

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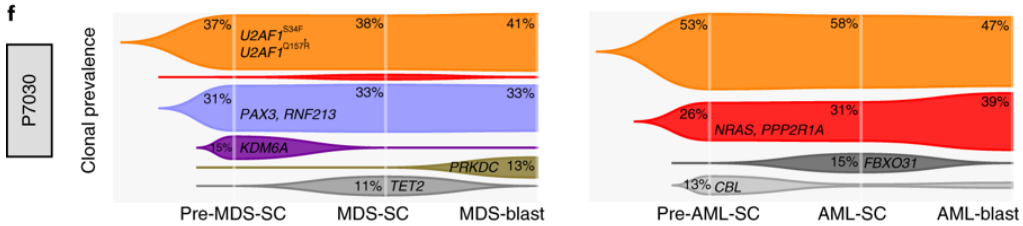
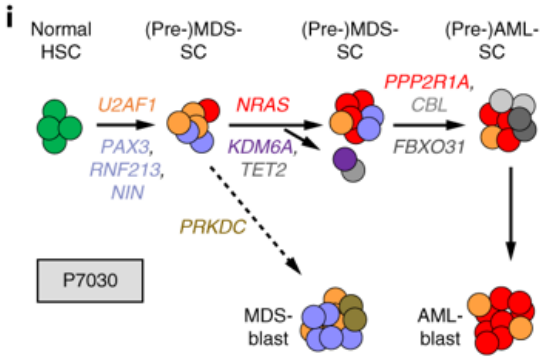
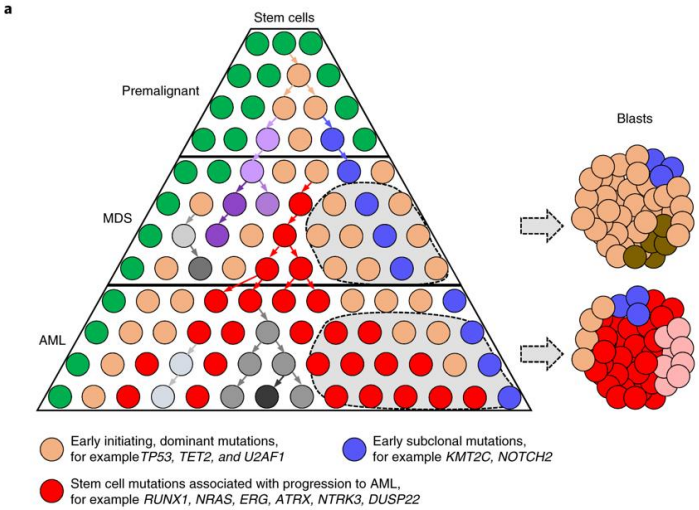
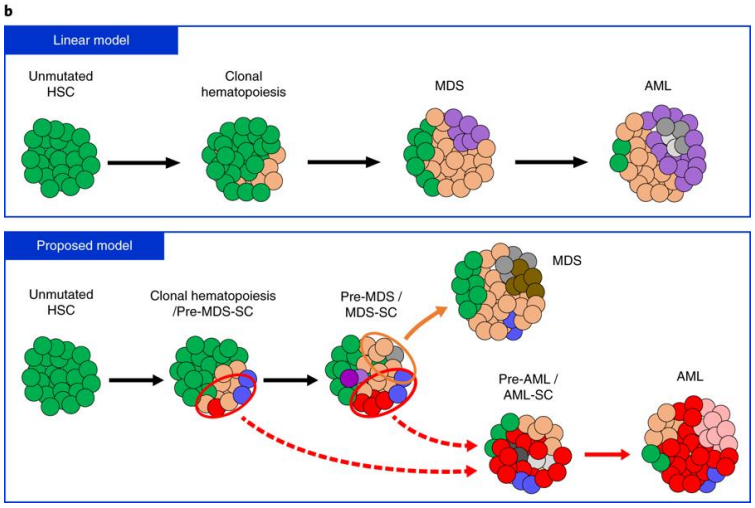


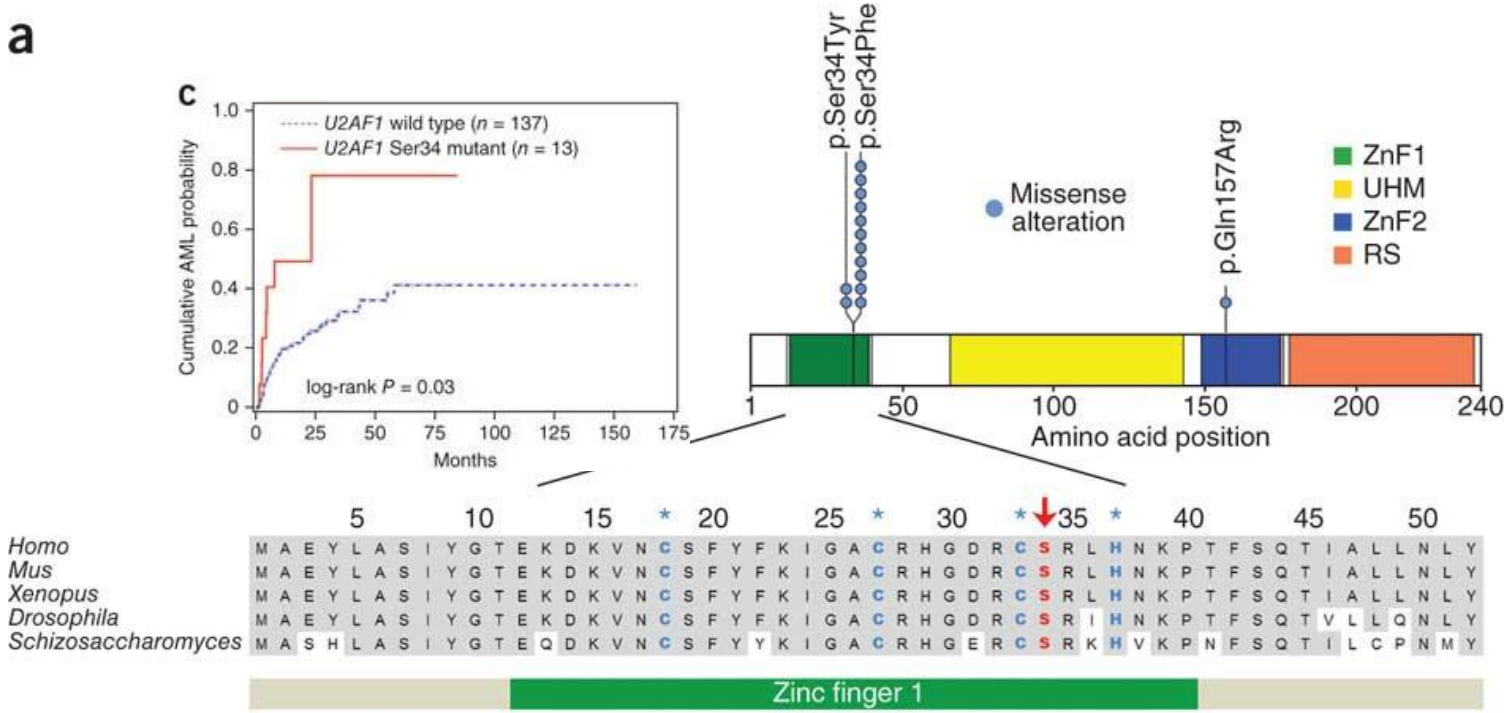






- Myelodysplasia / acute myeloid leukemia show tremendous heterogeneity.
  - Spliceosome mutations (at least U2AF1) seem to be early mutations.

Creation of the U2AF1-S34F point mutation at the endogenous locus



4 gene edited cell lines:

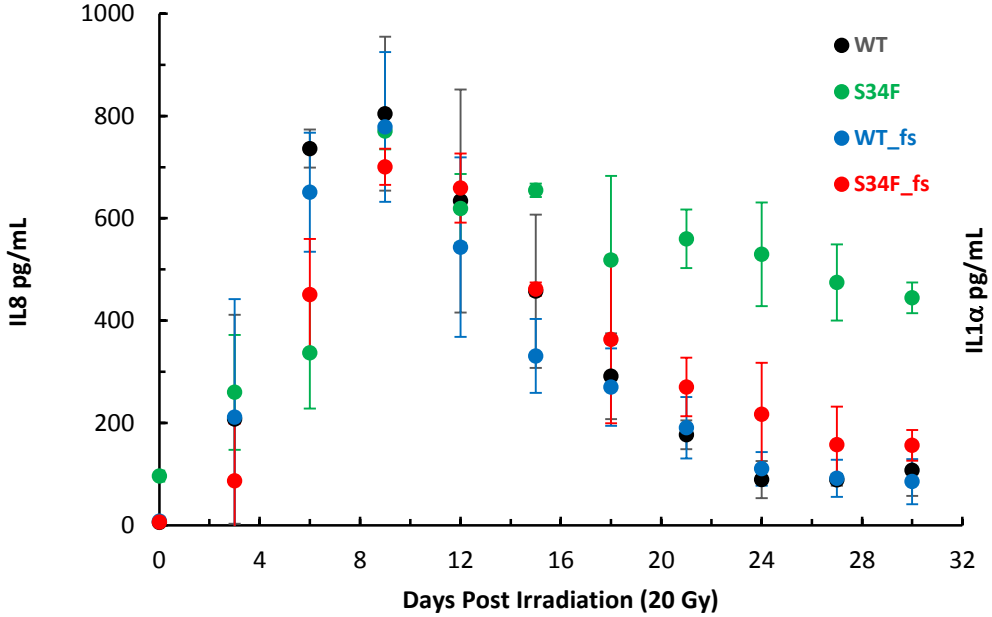
wt/wt ("wild-type")

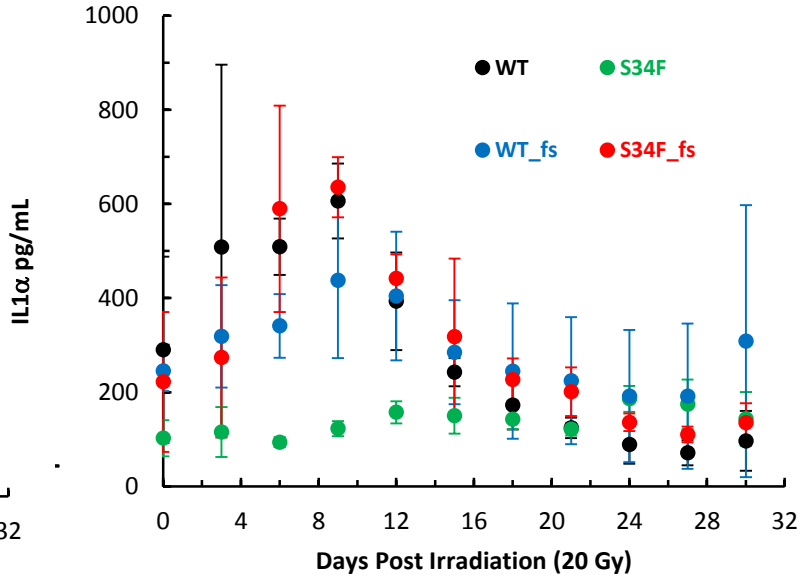
wt/fs ("frame shift")

wt/S34F ("mutant")

wt/fs ("rescue")

U2AF1 S34F cells survive high dose (20 Gy) irradiation, become senescent, and live for > 1 month in culture… and secrete interleukin 8



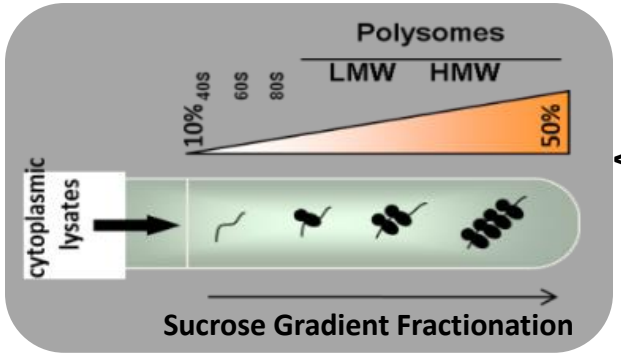


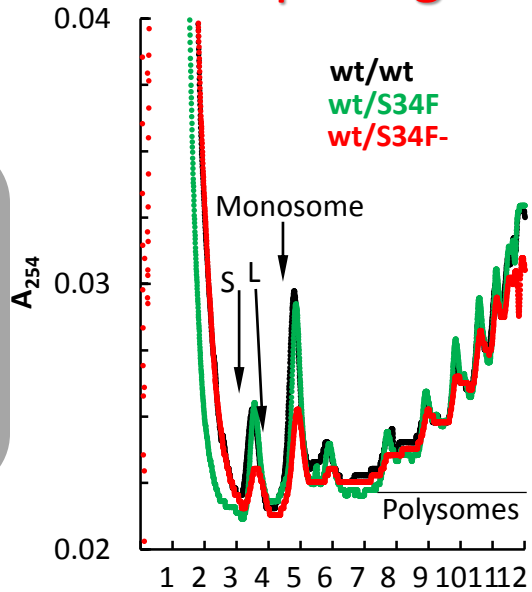
<u>ELISA</u> : IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IFNγ, TNFα . Cells = ~150, 000.
In HBECs, only IL-1a & IL-8 were detected in the medium

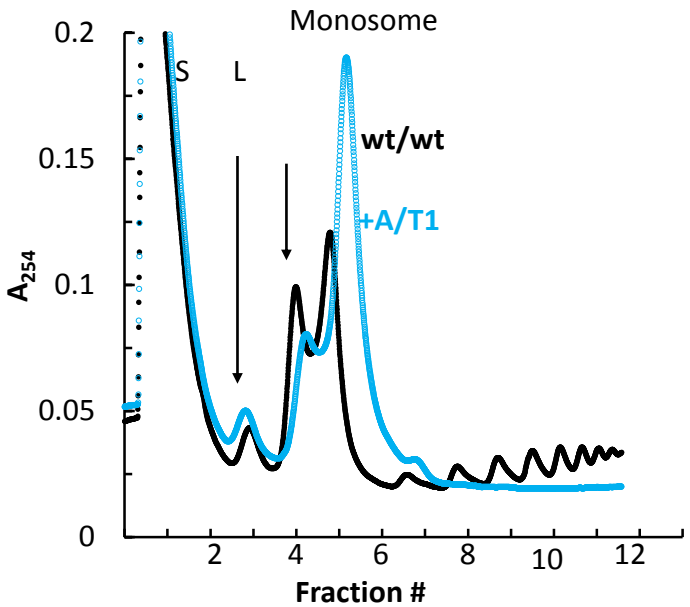
Interleukin 8 → angiogenesis, epithelial to mesenchymal transition, innate immune cell infiltration and a 'negative' microenvironment.

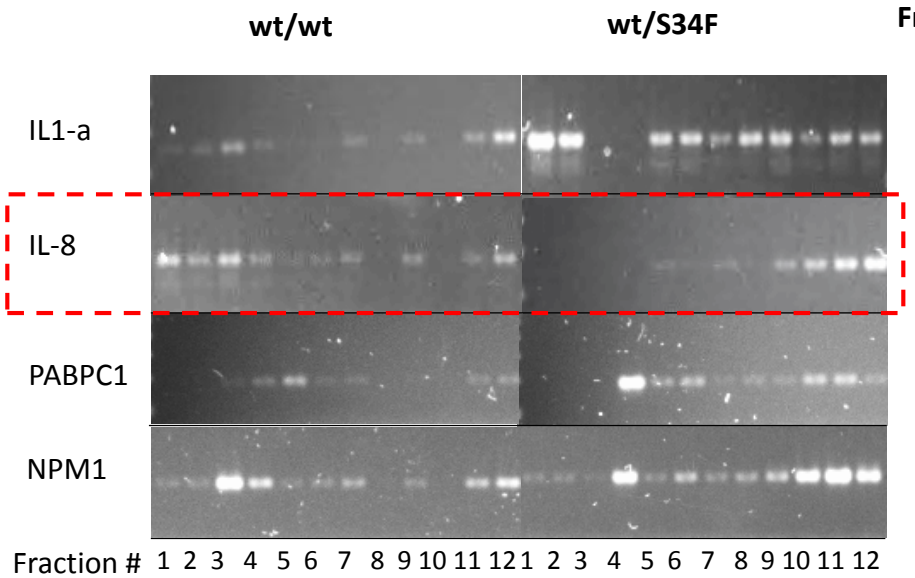
IL8 shows translational changes but no changes in mRNA abundance or splicing

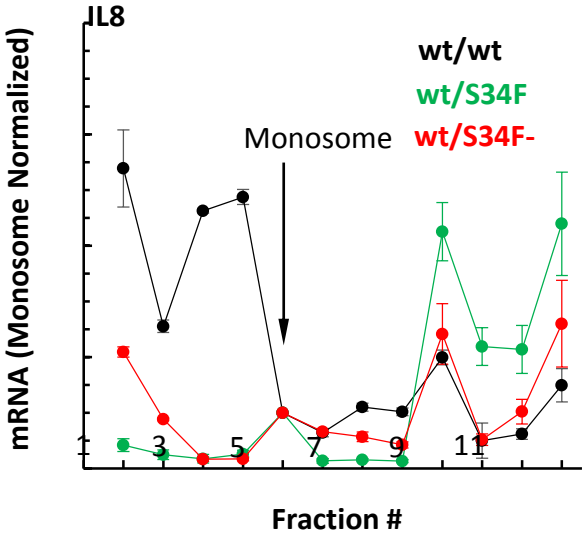
Polysome profiling



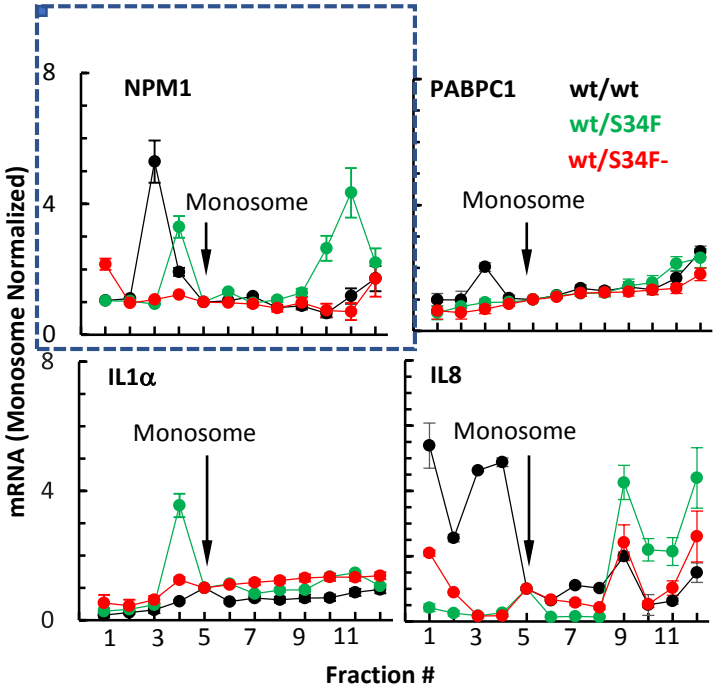


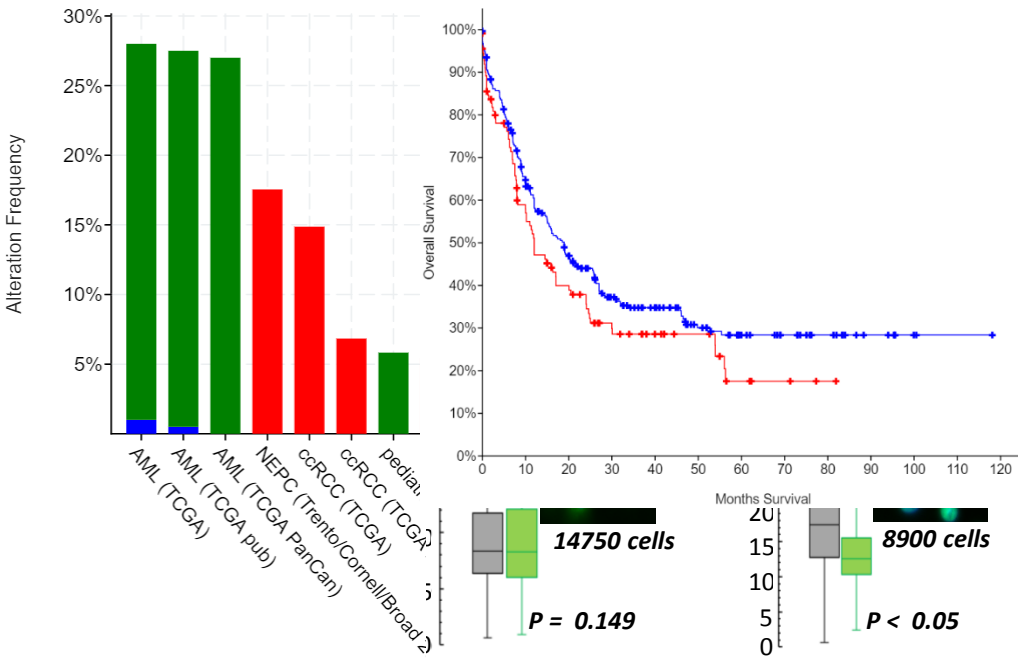




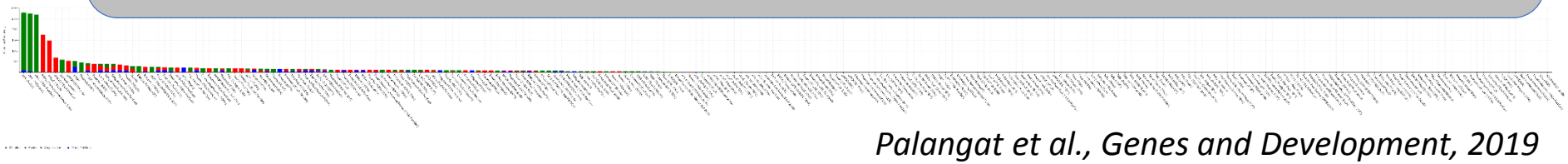


Polysome sequencing reveals hundreds of mRNA which show translational up-regulation in the U2AF1 S34F mutant cells



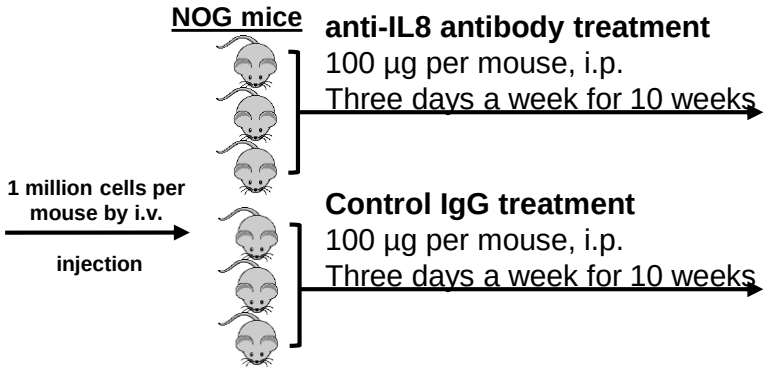


Npm1 → involved in ribosome biogenesis, trafficking, rRNA processing and frequently and <u>uniquely</u> altered in myeloid malignancy

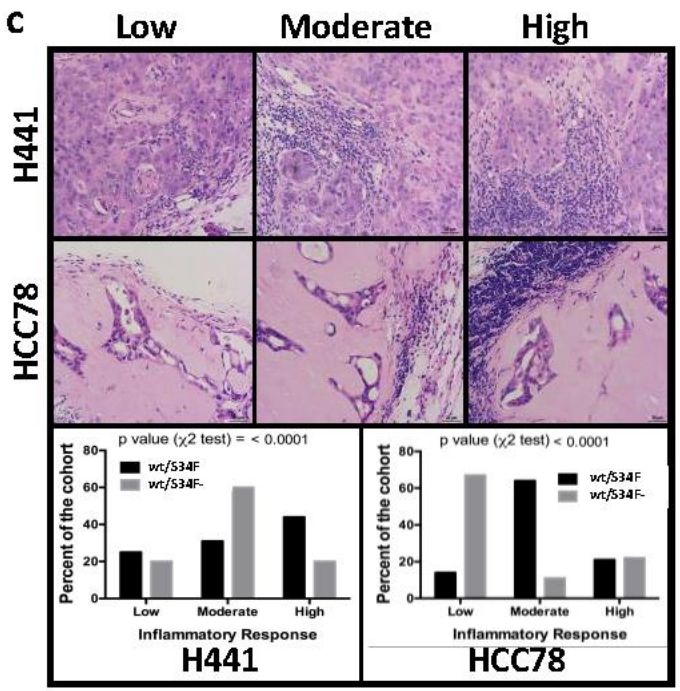


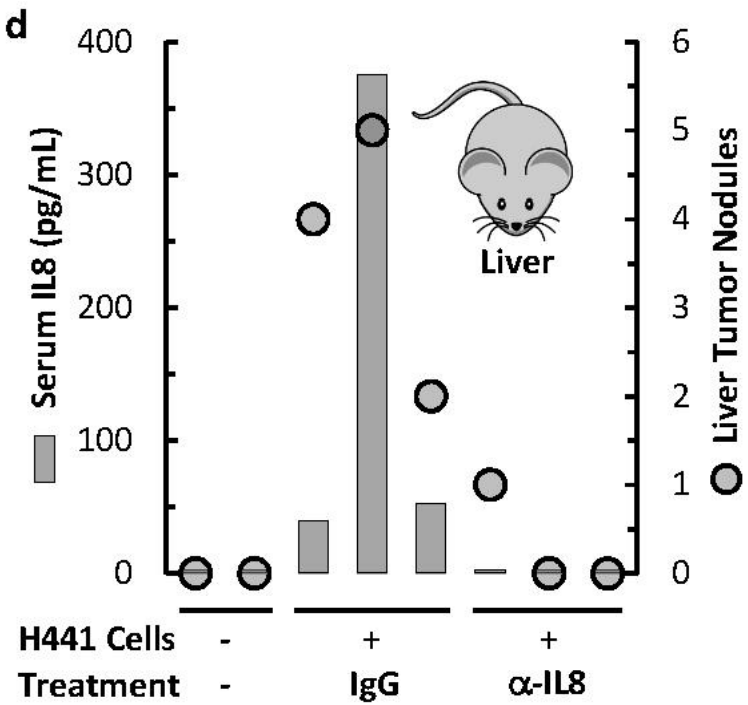
Palangat et al., Genes and Development, 2019

U2AF1 S34F results in increased inflammation, and blocking IL-8 with neutralizing Ab decreases tumor burden



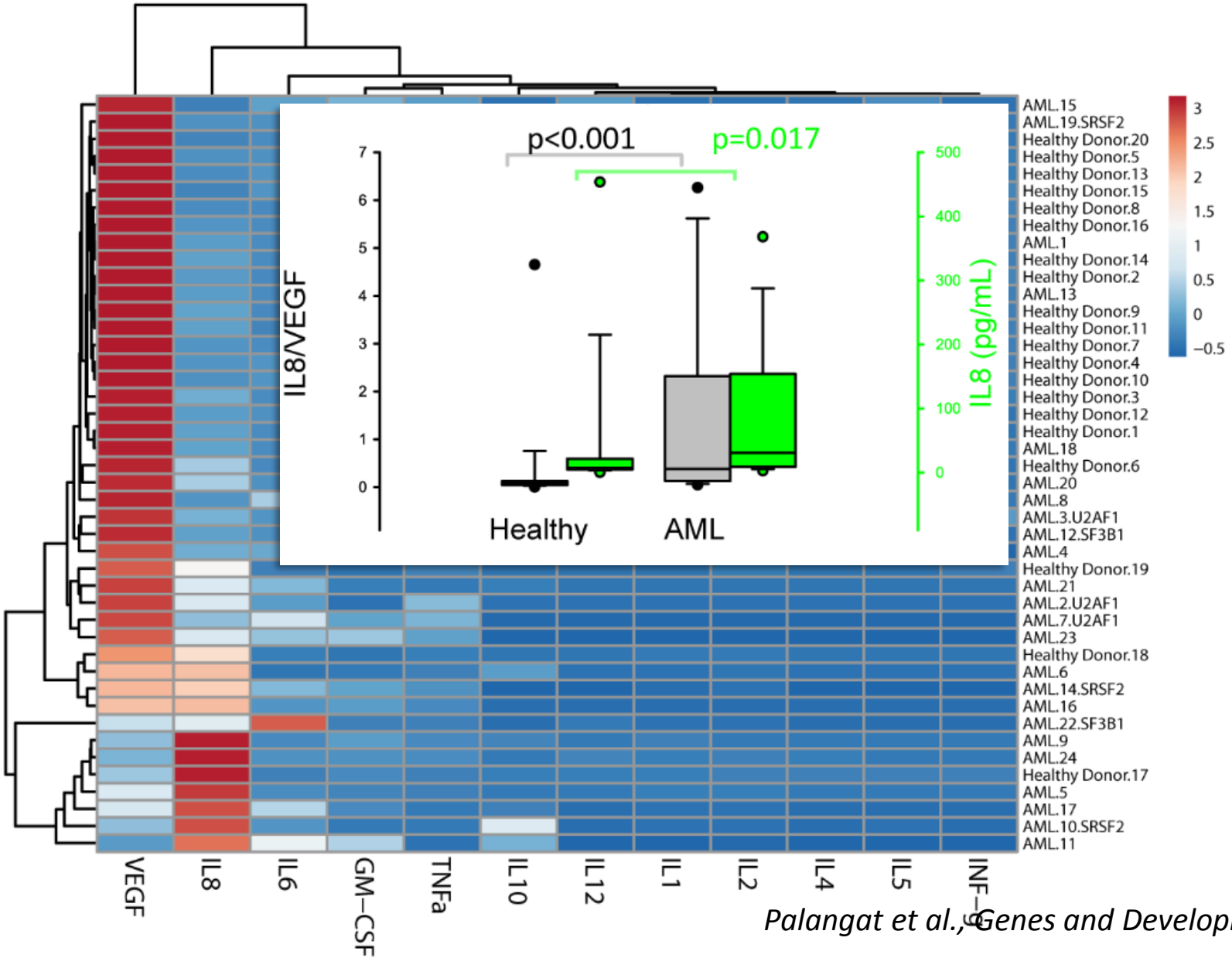
Assess inflammation and tumor growth





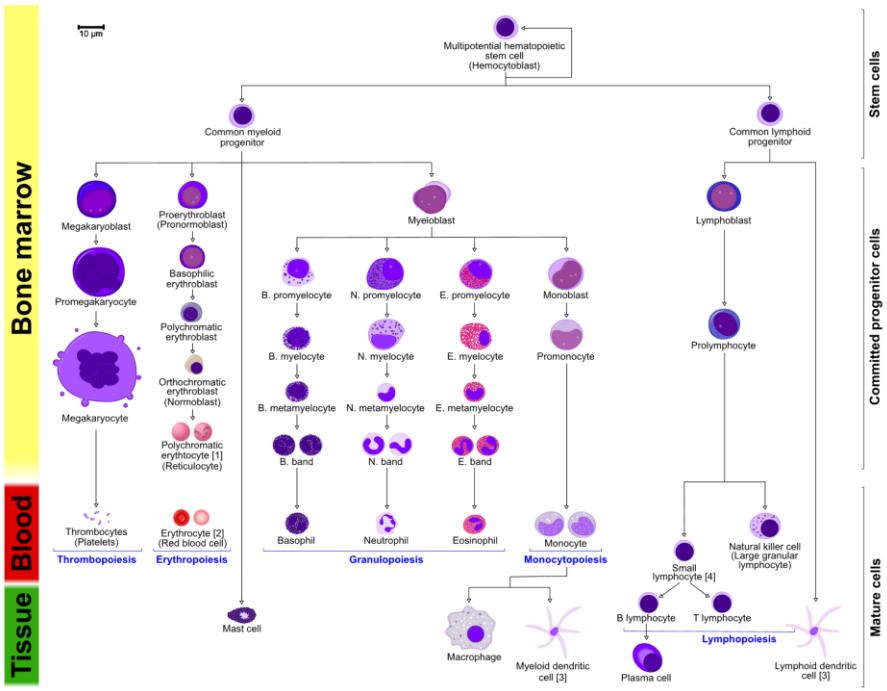
- anti-IL8 antibody: clone MAB208, R&D systems
- Control IgG: Mouse IgG1, R&D systems

IL-8 correlates with relapsed /refractory human AML, and splicing factor mutations are present in ~ 30% of patients

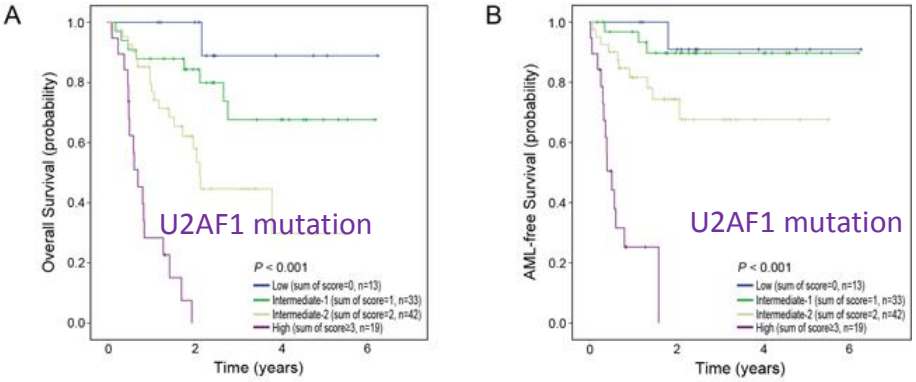


Palangat et al., Genes and Development, 2019

‘Splicing sickness’ or ‘ribosompathy’?



Hypothesis:
Translational regulation is important in balancing growth, proliferation, differentiation in HSCs and is altered by U2AF1 mutations (c.f. Diamond-Blackfan Anemia, Shwachman-Diamond Syndrome, 5q MDS).



Jung, Oncotarget, 2016

Palangat et al., Genes and Development, 2019

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Intramural Research Program

Our Research Changes Lives

Translational apparatus of the U2AF1 S34F cells is severely mis-regulated

cluster	gene	WT	S34F	FS	log2(wt/wt)	log2(s34f/wt)	log2(fs/wt)	Ribosomapthy
ribosome biogenesis	LTV1	3.92	10.07	6.79	0	1.361138124	0.79255792	
trans-acting factors	DDX18	4.84	11.27	5.31	0	1.219408563	0.133704814	
leucine tRNA recruitment	EIF2A	2.76	5.55	4.2	0	1.007819504	0.605721061	
trans-acting factors	RPF2	4.08	7.85	5.85	0	0.944123502	0.519867472	
ribosome biogenesis	NPM1	4.66	8.84	5.19	0	0.923716415	0.155404584	
Termination	ETF1	5.07	9.54	5.12	0	0.912003519	0.014158063	
RNA assistance by eIF3	EIF3J	5.19	9.57	5.71	0	0.882784386	0.137756207	
Ternary Complex	EIF2S1	6.66	11.97	7.18	0	0.84582907	0.108461667	
Cap binding	EIF4E	3.95	6.86	4.02	0	0.796355923	0.025342848	
RNA assistance by eIF3	EIF3M	4.57	7.83	4.89	0	0.776818142	0.0976403	
Termination	GSPT2	5.28	9.04	6.78	0	0.775784843	0.360747344	
Ribosome subunit joining	EIF5B	6.54	10.86	5.33	0	0.731661562	-0.295155103	
eIF2 exchange	EIF2B3	6.19	10.17	6.98	0	0.716308365	0.173287627	
RNA Polymerase I	POLR1B	5.13	8.4	5.04	0	0.711430502	-0.025535092	
Ribosome subunit joining	EIF5	5.88	9.62	6.03	0	0.710220739	0.036341847	
maturation	SBDS	5.1	8.31	4.85	0	0.70435123	-0.07251[illegible]5	SDS
Ternary Complex	EIF2S2	5.76	9.37	5.17	0	0.701980236	-0.155904531	
Ternary Complex	EIF2S3	3.63	5.8	3.47	0	0.676083352	-0.065033885	
RNA assistance by eIF3	EIF3E	3.51	5.57	3.69	0	0.666206297	0.072149786	
Start codon accuracy	EIF1AX	6.12	9.06	5.36	0	0.565979397	-0.191298652	
RNA assistance by eIF3	EIF3H	3.88	5.6	3.56	0	0.529370175	-0.124179411	
re-initiation factor	DENR	3.37	4.82	3.89	0	0.516284555	0.207021564	
eIF2 exchange	EIF2B1	6.03	8.6	6.65	0	0.512178658	0.141196338	
trans-acting factors	DKC1	4.18	5.85	5.56	0	0.484933682	0.411581941	
Elongation	EEF1A1	2.45	3.4	2.21	0	0.472752997	-0.14873538	
Helicase activity	EIF4A2	4.7	6.48	3.99	0	0.463333056	-0.23627201	
60 S	RPL7	3.33	4.43	2.97	0	0.411784521	-0.165059246	
RNA Polymerase I	POLR3A	4.64	6.15	4.32	0	0.406461605	-0.103093493	
CAP translation inhibition	EIF4EBP3	2.74	3.62	4.39	0	0.401813804	0.680045047	
Helicase activity	EIF4A3	6.57	8.5	7.15	0	0.371569471	0.122049871	
CAP translation inhibition	EIF4EBP2	3.04	3.93	3.04	0	0.370457989	0	
60 S	RPL5	3.25	4.2	3.02	0	0.36994961	-0.1058911[illegible]9	DBA
RNA assistance by eIF3	EIF3A	7.6	9.77	6.58	0	0.362359144	-0.207911835	
trans-acting factors	NOP56	4.83	6.16	4.87	0	0.350907162	0.011898583	
40 S progression	RPS12	2.11	2.69	2.11	0	0.350363174	0	
60 S	RPL21	3.19	4.04	3.47	0	0.340798869	0.121379239	
60 S	RPL6	3.52	4.44	3.14	0	0.334984248	-0.16481087	
60 S	RPL4	2.65	3.28	2.66	0	0.307703455	0.005433886	
RNA assistance by eIF3	EIF3L	2.67	3.28	2.62	0	0.296856073	-0.02727293	
trans-acting factors	RCL1	4.97	6.03	5.56	0	0.27891215	0.161839031	
eIF2 exchange	EIF2B2	5.87	7.11	7.67	0	0.276489057	0.385866074	
Helicase activity	EIF4B	3.31	3.9	2.8	0	0.236642907	-0.24140439	
RNA assistance by eIF3	EIF3D	4.3	4.95	4.37	0	0.203091865	0.02329662	
trans-acting factors	PDCD11	5.32	6.09	4.62	0	0.195015982	-0.203533394	
40 S initiation	RPS3A	3.16	3.56	2.94	0	0.171952683	-0.104108403	
re-initiation factor	MCTS1	3.4	3.68	3.99	0	0.11417102	0.230854	
leucine tRNA recruitment	EIF2D	2.91	3.13	2.76	0	0.105143504	-0.076350886	
60 S	RPL22	3.47	3.73	2.78	0	0.104239968	-0.31985078	
60 S	RPL17	2.91	3.12	2.69	0	0.100526876	-0.1134129[illegible]	
60 S	RPL31	2.95	3.16	2.51	0	0.099209604	-0.2330275[illegible]	DBA
Helicase activity	EIF4H	7.22	7.67	7.57	0	0.087227741	0.068294463	
40 S progression	RPS27A	3.15	3.34	2.51	0	0.084496274	-0.327664464	
	EIF4E2	5.19	5.48	5.49	0	0.078441355	0.081071611	
eIF2 exchange	EIF2B4	5.79	6.11	6.8	0	0.077609032	0.231971398	
40 S progression	RPSA	2.37	2.49	2.43	0	0.071258683	0.036069255	
40 S progression	RPS21	2.78	2.92	2.58	0	0.070883486	-0.107713817	
RNA assistance by eIF3	EIF3B	7.41	7.78	7.44	0	0.070296613	0.005829079	
40 S initiation	RPS28	3.19	3.33	2.83	0	0.061965753	-0.17275437[illegible]1	DBA
60 S	RPL39	3.01	3.14	2.8	0	0.061001072	-0.1043366[illegible]	
40 S progression	RPS29	2.85	2.95	2.79	0	0.049753035	-0.0306967[illegible]	DBA
40 S progression	RPS20	2.94	3.02	2.62	0	0.038732394	-0.166249343	
40 S progression	RPS25	2.95	3.03	2.72	0	0.038602839	-0.117108303	
40 S initiation	RPS27L	3.75	3.84	3.69	0	0.034215715	-0.023269779	
RNA assistance by eIF3	EIF3I	6.32	6.4	6.77	0	0.018147347	0.0992312[illegible]	
40 S initiation	RPS24	3.82	3.84	3.44	0	0.007533673	-0.1511640[illegible]	DBA
trans-acting factors	NOP2	5.31	5.33	5.16	0	0.005423672	-0.041340795	
40 S initiation	RPS23	2.73	2.71	2.32	0	-0.0106081	-0.234776146	
RNA Polymerase I	POLR1A	5.86	5.81	5.12	0	-0.012362501	-0.194756854	
60 S	RPL23A	2.62	2.59	2.4	0	-0.016614714	-0.1265324[illegible]	
60 S	RPL11	3.14	3.04	2.67	0	-0.046693235	-0.2339248[illegible]7	DBA
trans-acting factors	NOP10	3.2	3.09	3.28	0	-0.050465067	0.03562391	
40 S initiation	RPS6	3.37	3.25	3.07	0	-0.052308873	-0.1345099[illegible]5	DBA
60 S	RPL35A	3.67	3.53	3.4	0	-0.05611188	-0.1102453[illegible]	DBA
40 S progression	RPS2	2.29	2.2	2.42	0	-0.057844075	0.079659[illegible]	
60 S	RPL38	3.42	3.28	2.8	0	-0.06030051	-0.288569498	
60 S	RPL37A	2.64	2.51	2.49	0	-0.072850565	-0.084392187	
Cap binding	EIF4G1	6.61	6.27	5.66	0	-0.076184829	-0.223848219	
eIF2 exchange	EIF2B5	6.19	5.86	5.34	0	-0.079038745	-0.213099668	
60 S	RPL9	3.49	3.28	2.89	0	-0.089531222	-0.272157544	
40 S initiation	RPS27	2.69	2.51	2.46	0	-0.099918809	-0.128947857	
60 S	RPL13A	2.54	2.37	2.28	0	-0.099941438	-0.155794673	
60 S	RPL36A	3.32	3.06	2.89	0	-0.117651589	-0.200113749	
Ribosome subunit joining	EIF6	7.14	6.55	7.65	0	-0.124429168	0.099535674	
40 S initiation	RPS7	4.29	3.92	3.57	0	-0.130123993	-0.2650535[illegible]3	DBA
60 S	RPL14	4.02	3.66	3.87	0	-0.135351853	-0.054861935	
60 S	RPL12	3.51	3.17	3.39	0	-0.14698819	-0.050185757	
60 S	RPL36	3.23	2.91	2.8	0	-0.150515012	-0.206107338	
60 S	RPL41	2.27	2.04	2.3	0	-0.154123145	0.018941564	
RNA assistance by eIF3	EIF3F	3.26	2.9	3.26	0	-0.168819064	0	
40 S progression	RPS19	3.26	2.88	2.79	0	-0.178803153	-0.2246068[illegible]2	DBA
trans-acting factors	FBL	3.75	3.31	4.32	0	-0.180059379	0.204140717	
Start codon accuracy	EIF1	4.74	4.18	4.51	0	-0.181384117	-0.071759626	
60 S	RPL30	3.5	3.08	3.17	0	-0.184424571	-0.142872082	
60 S	RPL23	3.28	2.88	2.93	0	-0.187627003	-0.1627951[illegible]	
40 S progression	RPS10	3.17	2.78	3.33	0	-0.189397957	0.0710393[illegible]7	DBA
40 S initiation	RPS13	3.78	3.31	3.38	0	-0.191555017	-0.161362988	
60 S	RPL34	3.41	2.98	3.16	0	-0.194459409	-0.109847181	
40 S initiation	RPS4X	3.37	2.94	3.46	0	-0.196932436	0.038023446	
60 S	RPL37	3.59	3.11	3.4	0	-0.207069264	-0.078449098	
40 S progression	RPS18	3.28	2.83	2.72	0	-0.212893762	-0.270089163	
40 S initiation	RPS5	3.09	2.66	2.61	0	-0.216180592	-0.243557031	
trans-acting factors	RRS1	7.01	6.01	6.58	0	-0.222049453	-0.09132686	
60 S	RPL26	3.63	3.11	3.4	0	-0.223054968	-0.0944348[illegible]2	DBA
60 S	RPL19	3.99	3.41	3.6	0	-0.226617007	-0.14839184	
60 S	RPL27A	3.42	2.92	2.94	0	-0.228027956	-0.21818017	
RNA assistance by eIF3	EIF3K	4.58	3.91	5.11	0	-0.228178991	0.157975693	
60 S	RPL24	4.76	4.06	4.26	0	-0.229481846	-0.160108143	
60 S	RPL29	3.23	2.75	2.77	0	-0.232102546	-0.221648189	
60 S	RPL10A	3.96	3.37	3.73	0	-0.232751839	-0.0863248	
	RBM4	6.5	5.5	5.16	0	-0.2410081	-0.333068652	
40 S initiation	RPS14	3.76	3.18	3.22	0	-0.241705896	-0.2236719[illegible]4	5q-MDS
Elongation	EEF2	2.84	2.4	2.55	0	-0.242856524	-0.155393683	
60 S	RPL3	3.48	2.94	3.01	0	-0.243271151	-0.209323819	
60 S	RPL10	3.16	2.66	2.72	0	-0.248498313	-0.216317907	
CAP translation inhibition	EIF4EBP1	5.02	4.18	5.33	0	-0.264184422	0.086448169	
60 S	RPL13	3.25	2.7	2.77	0	-0.267480311	-0.230553742	
40 S initiation	RPS11	2.59	2.15	2.4	0	-0.268615438	-0.10991769[illegible]	
40 S progression	RPS26	5.24	4.34	5.4	0	-0.271871769	0.0433925[illegible]5	DBA
60 S	RPL27	4.47	3.7	4.73	0	-0.272749561	0.081565352	
60 S	RPL15	4.05	3.35	3.71	0	-0.273760812	-0.1265027[illegible]1	DBA
40 S progression	RPS3	3.42	2.82	3.31	0	-0.278301162	-0.047165108	
40 S initiation	RPS8	3.7	3.05	3.32	0	-0.278716028	-0.156342029	
40 S initiation	RPS16	3.15	2.56	2.78	0	-0.299208018	-0.180266946	
60 S	RPL8	3.48	2.82	3.16	0	-0.303392143	-0.139162748	
40 S initiation	RPS9	3.33	2.69	3.01	0	-0.307916005	-0.14575869	
60 S	RPL35	3.94	3.16	3.83	0	-0.318271071	-0.0408512[illegible]3	DBA
60 S	RPL7A	3.65	2.92	3.15	0	-0.321928095	-0.21254463[illegible]	
40 S progression	RPS17	3.65	2.86	3.34	0	-0.351881317	-0.1280483[illegible]1	DBA
40 S progression	RPS15	4.09	3.2	3.71	0	-0.354028938	-0.140681656	
40 S initiation	RPS15A	3.8	2.95	3.17	0	-0.365284464	-0.261516578	
60 S	RPL18A	3.41	2.63	2.93	0	-0.37470894	-0.218871075	
60 S	RPL18	3.75	2.87	3.07	0	-0.385839859	-0.28865194	
RNA assistance by eIF3	EIF3G	4.65	3.51	4.41	0	-0.405759686	-0.07645206	
trans-acting factors	PES1	6.64	5.01	7.07	0	-0.406372638	0.090526973	
60 S	RPL28	3.13	2.26	3.07	0	-0.469839885	-0.027924002	
60 S	RPL32	4.34	3.11	3.88	0	-0.480780462	-0.16163839	
trans-acting factors	DDX54	7.02	5.01	7.65	0	-0.486660427	0.123988717	
trans-acting factors	BOP1	6.88	4.71	6.63	0	-0.546681505	-0.053399695	
RNA assistance by eIF3	EIF3C	5.67	3.75	6.76	0	-0.59645814	0.253674511	

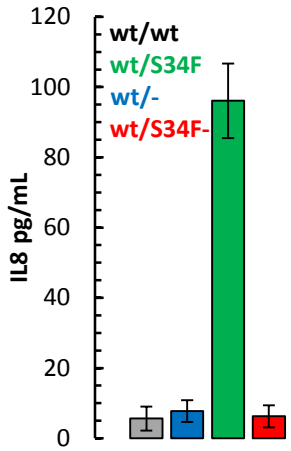
Diamond-Blackfan Anemia

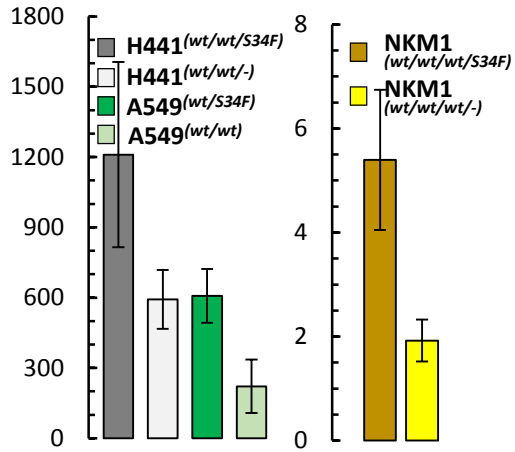
Shwachman-Diamond Syndrome

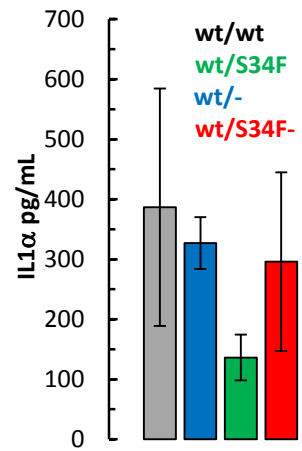
5q-MDS

Palangat et al., Genes and Development, 2019

U2AF1 S34F cells show pronounced interleukin 8 secretion even before irradiation, and this effect is observed in human cancer cells







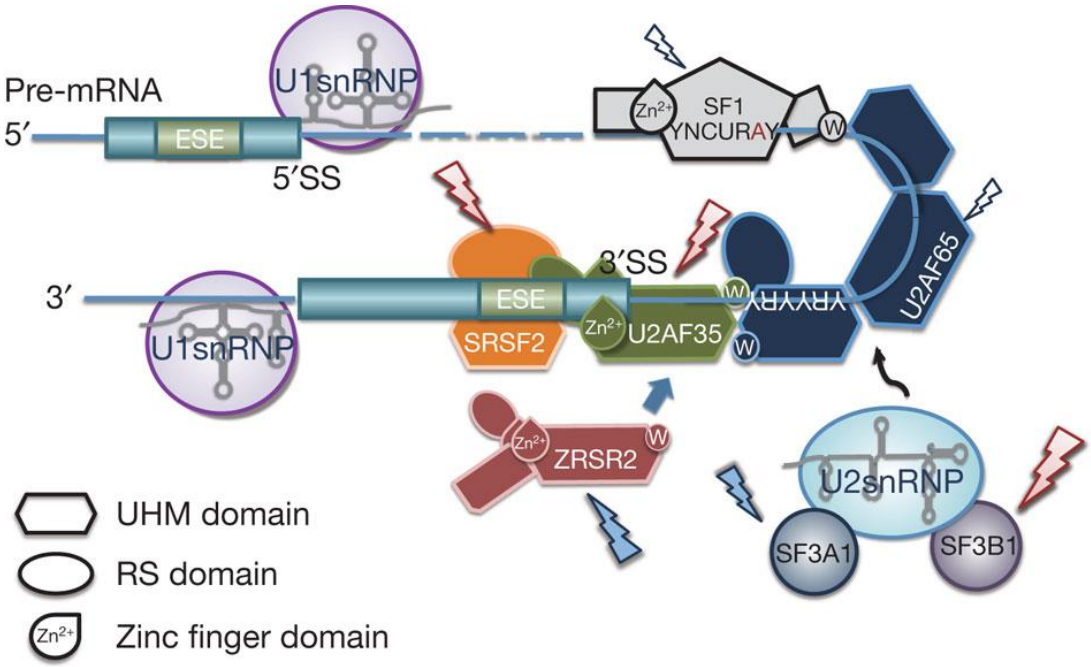
Interleukin-8 is ~ 20x Up-regulated even before DNA damage.

The same trend is visible in human cancer cell lines that have been corrected by CRISPR.

Interleukin 1a shows the opposite effect to interleukin 8.

Palangat et al., Genes and Development, 2019

Recurrent somatic mutations in splicing factors in a variety of cancers



Myelodysplastic syndrome
Chronic lymphocytic leukemia
Acute myeloid leukemia
Hairy cell leukemia
Breast cancer
Lung adenocarcinoma
Uveal melanoma

K Yoshida *et al*. *Nature* , 1-6 (2011) doi:10.1038/nature10496

Many mutations in factors (e.g. *SF3B1, U2AF1*) involved in 3’ SS or branchpoint recognition.