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Supplementary Fig. 1

Differentiation potential of CD38⁺ HSPCs in SM. (a) Gating strategy used for sorting CMPs, GMPs, and MEPs. (b) Distribution of granulocyte/monocyte, erythroid, or mixed colonies, counted on days 12-13. (c) Representative colonies. CMPs, GMPs, and MEPs were sorted from samples SM21, SM22, and SM23; whereas CMPs and GMPs were sorted from SM20. Images were captured using a Zeiss AxioVert 200 Inverted microscope (Zeiss, Oberkochen Germany).

Supplementary Fig. 2

SM subjects are similar to the controls but differ from MDS/MPN subjects. (a) Percentage of HSPCs per total CD34⁺ cells comparing the control, ISM, AdvSM groups with the MDS/MPN subjects. (b) The percentage of CD34⁺ cells in controls, including CM (open circles), ISM and AdvSM and MDS/MPN subjects. Means and SEM are shown. Unpaired one-way ANOVA with Tukey's comparisons were used in panel a and b, ** $P < 0.01$, *** $P < 0.001$.

Supplementary Fig. 3

Expression profile of mast cells and CMP^{FcεRI+} cells in PB and BM.

(a) Representative gating of MCPs in PB showing CD123, CD45RA and CD34 expression. (b) Backgating analysis of CMPs^{FcεRI+} and mature mast cells in BM of a representative subject. CMPs^{FcεRI+} and mature mast cells are overlaid in the bottom panels.

Supplementary Fig. 4

CD133 and CD117-expression on HSPCs. (a) CD117 expression on CMP^{FcεRI+} cells was lower than on CMP^{FcεRI-} cells in 6 randomly selected samples, showing the median fluorescent intensity of CD117 expression. (b) CD133 expression on HSPCs from samples Ctrl113, SM19, and SM36, showing the median fluorescent intensity for the stained sample minus the FMO control. Expression levels were normalized within each sample by assigning the sum of the expression of all populations to as 100%.

Supplementary Fig 5.

Staining for granularity and tryptase in cultured CMP^{FcεRI-}, CMP^{FcεRI+} and GMP cells.

Representative images of cultured cells from Ctrl29 and SM33 on days 17-19. Images were captured using an Olympus XC10 camera (Olympus, Tokyo, Japan). The image width corresponds to 29 μm.

Supplementary Fig. 6

The single-cell mutation assay shows high specificity and sensitivity in the mast cell lines HMC-1.2 and ROSA^{KIT WT}. (a) Method of single-cell analysis in the mast cell lines ROSA^{KIT WT} and HMC-1.2 (*KIT* D816V). Cells were labelled separately for CD117 and sorted using the index sort option. Each cell was analysed for a control assay and mutation assay. Mutation data were linked to the index data. Bulk cell sorting was used as a control for the multiplex qPCR. (b) Flow diagram of the quality control performed for the single cells after qPCR. (c) Numbers and percentages of cells that showed amplification in the control assay for the wildtype and mutated cells. (d) Numbers and percentages of cells that showed amplification in the mutation assay.

Supplementary Fig. 7

Details of single sorted HSPCs in bone marrow and peripheral blood. (a) All single sorted cells identified by using the index-sorting data provided for each patient as a percentage of the total number of sorted cells. (b) Numbers and percentages of mutated cells in all HSPC populations including peripheral blood mast cell progenitors (PB MCP) in patient SM16. (c) FcεRI and CD117 expression on LMPPs in SM14.

Supplementary Table 1. Patient characteristics.

Patient	Gender	Age	WHO classification	Major criterion	Tryptase, ng/mL	D816V, PB	D816V, BM	Atypical MC morphology	CD2/ CD25	UP
Ctrl02	F	29	No SM	<i>NA</i>	<i>NA</i>	<i>NA</i>	<i>NA</i>	<i>NA</i>	<i>NA</i>	<i>NA</i>
Ctrl04	F	73	No SM	-	10	-	-	-	-	-
Ctrl24	F	42	No SM	<i>NA</i>	<i>NA</i>	<i>NA</i>	<i>NA</i>	<i>NA</i>	<i>NA</i>	<i>NA</i>
Ctrl26	M	37	No SM	-	45 †	-	-	-	-	-
Ctrl29	M	32	No SM	<i>NA</i>	<i>NA</i>	<i>NA</i>	<i>NA</i>	<i>NA</i>	<i>NA</i>	<i>NA</i>
Ctrl37	F	45	No SM	-	3.9	-	-	-	-	-
Ctrl05	F	35	CM *	-	7.6	-	-	-	-	+
Ctrl38	M	53	CM *	-	6	-	-	-	-	+
Ctrl113	M	21	CM *	-	6.3	-	-	-	-	+
SM07	F	53	ISM	+	65	+	+	+	+	+
SM08	F	49	ISM	-	14	+	+	+	+	+
SM09	F	65	ISM	+	42	+	<i>ND</i>	-	+	+
SM10	M	26	ISM	-	9.2	-	+	+	+	+
SM11	F	31	ISM	-	3.6	+	+	+	+	+
SM13	F	65	ISM	+	19	+	+	+	+	+
SM104	F	52	ISM	-	11	-	+	- ‡	+	-
SM105	F	70	ISM	+	24	+	+	+	+	+
SM19	F	86	ISM	-	15	+	+	+	+	+
SM20	M	58	ISM	-	23	<i>ND</i>	+	+	+	+
SM21	M	46	ISM	-	8.8	+	+	+	+	+
SM22	M	77	ISM	+	24	<i>ND</i>	+	<i>ND</i>	+	-
SM23	F	45	ISM	+	32	+	+	+	+	+
SM25	F	60	ISM	+	14	<i>ND</i>	+	+	+	-
SM28	F	56	ISM	+	24	<i>ND</i>	+	+	+	+
SM32	F	73	ISM §	+	88	+	+	+	+	+
SM33	M	25	ISM	<i>ND</i>	15	+	+	<i>ND</i>	+	+
SM36	F	46	ISM	-	14	+	+	+	+	+
SM39	F	65	ISM	-	49	+	+	-	+	+
SM06	M	85	ASM ¶	+	95	+	+	-	+	<i>ND</i>
SM14	M	44	ASM	+	80	+	+	-	+	+
SM16	M	46	ASM	+	125	<i>ND</i>	+	+	+	+
SM17	F	69	SM-AHN #	+	4.8	<i>ND</i>	+	+	+	-
SM18	M	67	SM-AHN #	+	43	<i>ND</i>	+	+	+	-
SM31	F	69	SM-AHN **	+	61	+ ††	+ ††	+	+	+
MDS12	F	72	MPN	-	18	-	-	+	+	<i>ND</i>
MDS30	M	78	MDS	-	17	-	-	-	+	-

NA, not applicable; *ND*, not determined; CM, cutaneous mastocytosis; UP, Urticaria pigmentosa; PB, peripheral blood; BM, bone marrow; † Patient has severe kidney failure; * Patient has cutaneous mastocytosis; ‡ Second biopsy showed atypical morphology; § Small CLL clone in bone marrow, no peripheral lymphocytosis; || Bone marrow biopsy not available; ¶ ASM-AHN suspected; # SM-MPN; ** SM-MDS-MPN; †† High mutation burden

Supplementary Table 2. Numbers of *KIT*-mutated and wildtype *KIT* haematopoietic stem and progenitor cells and mast cells.

	SM7		SM9		SM10		SM11		SM13		SM6		SM14		SM16		SM17		SM18		SM28		SM31	
	M	WT	M	WT	M	WT	M	WT	M	WT	M	WT	M	WT	M	WT	M	WT	M	WT	M	WT	M	WT
HSC	1	134	1	80	0	102	0	112	0	278	0	28	2	232	72	225	0	182	3	291	1	121	43	95
MPP	2	48	3	116	0	55	0	86	0	154	0	50	3	148	33	86	0	288	4	122	<i>ND</i>	<i>ND</i>	<i>ND</i>	<i>ND</i>
CMP ^{FcεRI-}	0	61	2	146	0	81	1	129	1	207	1	75	1	215	104	124	2	288	3	261	<i>ND</i>	<i>ND</i>	140	2
CMP ^{FcεRI+}	1	18	0	15	0	22	0	17	0	23	0	30	0	32	15	27	1	84	0	19	1	85	266	12
MEP	1	41	2	78	0	36	0	89	0	107	0	38	0	187	62	73	0	55	4	179	<i>ND</i>	<i>ND</i>	<i>ND</i>	<i>ND</i>
LMPP	2	49	2	38	0	47	0	90	1	88	1	43	4	71	11	33	0	22	0	72	<i>ND</i>	<i>ND</i>	<i>ND</i>	<i>ND</i>
GMP	5	131	8	213	1	217	1	161	1	349	2	268	4	227	190	213	1	357	6	292	2	141	76	61
CDP	1	30	3	33	0	26	1	19	0	26	0	62	0	46	15	30	0	63	2	73	<i>ND</i>	<i>ND</i>	<i>ND</i>	<i>ND</i>
MLP	0	1	0	1	0	3	0	1	0	1	0	0	0	8	0	2	0	2	0	2	<i>ND</i>	<i>ND</i>	<i>ND</i>	<i>ND</i>
B-NK	4	135	0	36	0	89	1	13	0	347	0	0	0	262	46	120	0	82	1	61	<i>ND</i>	<i>ND</i>	<i>ND</i>	<i>ND</i>
Mast cells	60	7	25	13	41	16	24	16	97	1	103	8	83	4	83	6	78	15	81	2	87	1	187	7

M, number of cells with the *KIT* D816V mutation; WT, number of cells with wildtype *KIT*; *ND*, not determined

Supplementary Table 3. Relations between clinical and experimental data.

	% Mutated mast cells	% Mutated HSPCs	Serum tryptase	Age
% Mast cells	$R^2 = .471$; $P = .0137$; $n = 12$	$R^2 = .016$; $P = .7265$; $n = 10$	$R^2 = .287$; $P = .0058$; $n = 25$	$R^2 = .068$; $P = .2095$; $n = 25$
% Mutated mast cells		$R^2 = .029$; $P = .6356$; $n = 10$	$R^2 = .339$; $P = .0469$; $n = 12$	$R^2 = .294$; $P = .0688$; $n = 12$
% Mutated HSPCs			$R^2 = .452$; $P = .0332$; $n = 10$	$R^2 = .013$; $P = .7510$; $n = 10$
Serum tryptase				$R^2 = .139$; $P = .0664$; $n = 25$

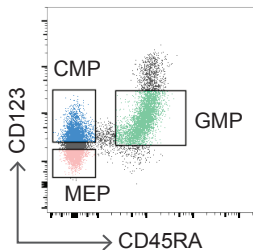
Supplementary Table 4. Percentage of mutation per haematopoietic stem and progenitor cells and mast cells.

	SM7	SM9	SM10	SM11	SM13	SM6	SM14	SM16	SM17	SM18	SM28	SM31
HSC	0.74	1.23	0.00	0.00	0.00	0.00	0.85	24.24	0.00	1.02	0.82	31.16
MPP	4.00	2.52	0.00	0.00	0.00	0.00	1.99	27.73	0.00	3.17	<i>ND</i>	<i>ND</i>
CMP ^{FcεRI-}	0.00	1.35	0.00	0.77	0.48	1.32	0.46	45.61	0.69	1.14	<i>ND</i>	98.59
CMP ^{FcεRI+}	5.26	0.00	0.00	0.00	0.00	0.00	0.00	35.71	1.18	0.00	1.16	95.68
MEP	2.38	2.50	0.00	0.00	0.00	0.00	0.00	45.93	0.00	2.19	<i>ND</i>	<i>ND</i>
LMPP	3.92	5.00	0.00	0.00	1.12	2.27	5.33	25.00	0.00	0.00	<i>ND</i>	<i>ND</i>
GMP	3.68	3.62	0.46	0.62	0.29	0.74	1.73	47.15	0.28	2.01	1.40	55.47
CDP	3.23	8.33	0.00	5.00	0.00	0.00	0.00	33.33	0.00	2.67	<i>ND</i>	<i>ND</i>
MLP	0.00	0.00	0.00	0.00	0.00	<i>ND</i>	0.00	0.00	0.00	0.00	<i>ND</i>	<i>ND</i>
B-NK	2.88	0.00	0.00	7.14	0.00	<i>ND</i>	0.00	27.71	0.00	1.61	<i>ND</i>	<i>ND</i>
Total CD34 ⁺	2.56	2.70	0.15	0.55	0.19	0.67	0.97	37.00	0.28	1.65	<i>ND</i>	<i>ND</i>
Mast cells	89.55	65.79	71.93	60.00	98.98	92.79	95.40	93.26	83.87	97.59	98.86	96.39

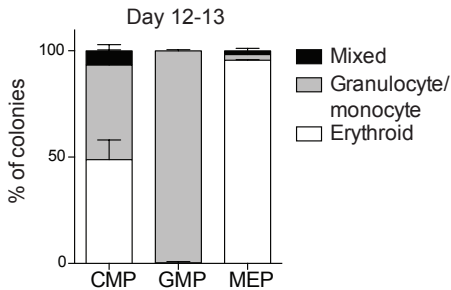
ND, not determined

Supplementary Fig. 1

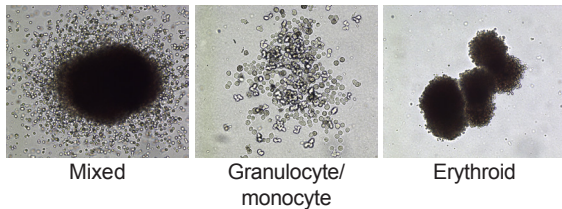
a



b

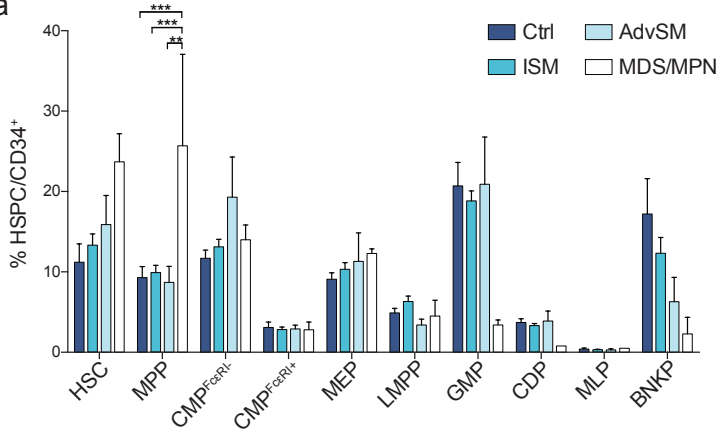


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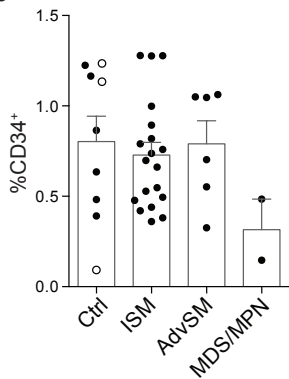


Supplementary Fig. 2

a



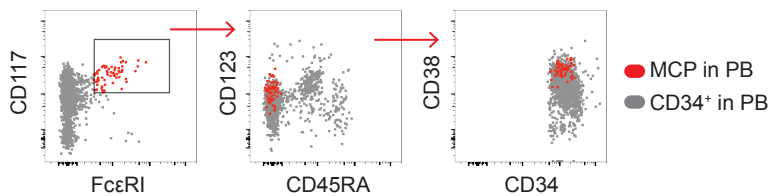
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Supplementary Fig. 3

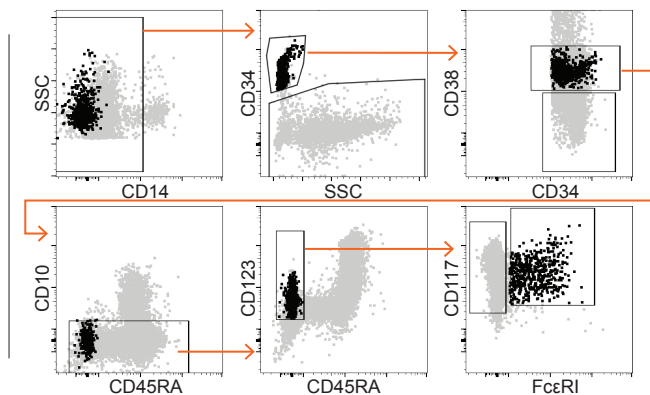
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SM105 (PB)

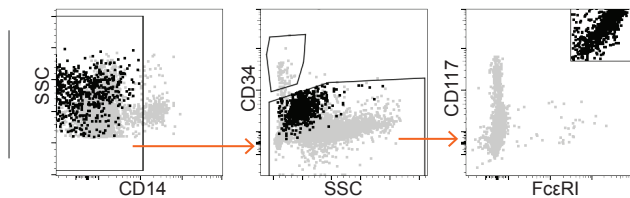


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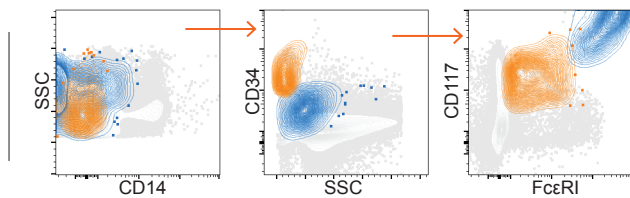
CMP^{FcεRI+}



Mast cells

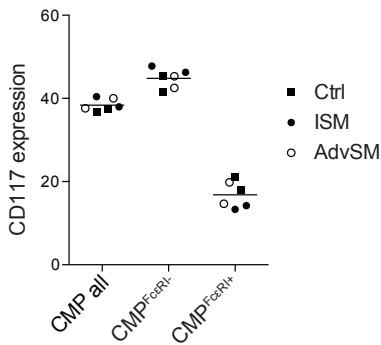


Mast cells
and
CMP^{FcεRI+}

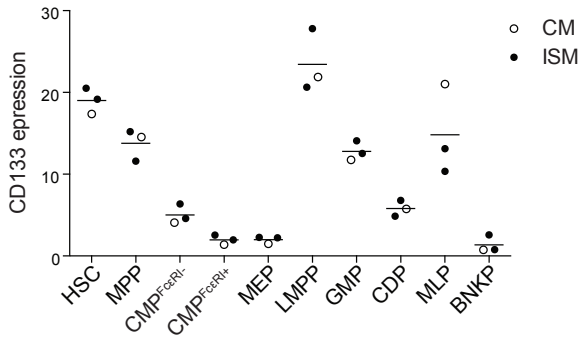


Supplementary Fig. 4

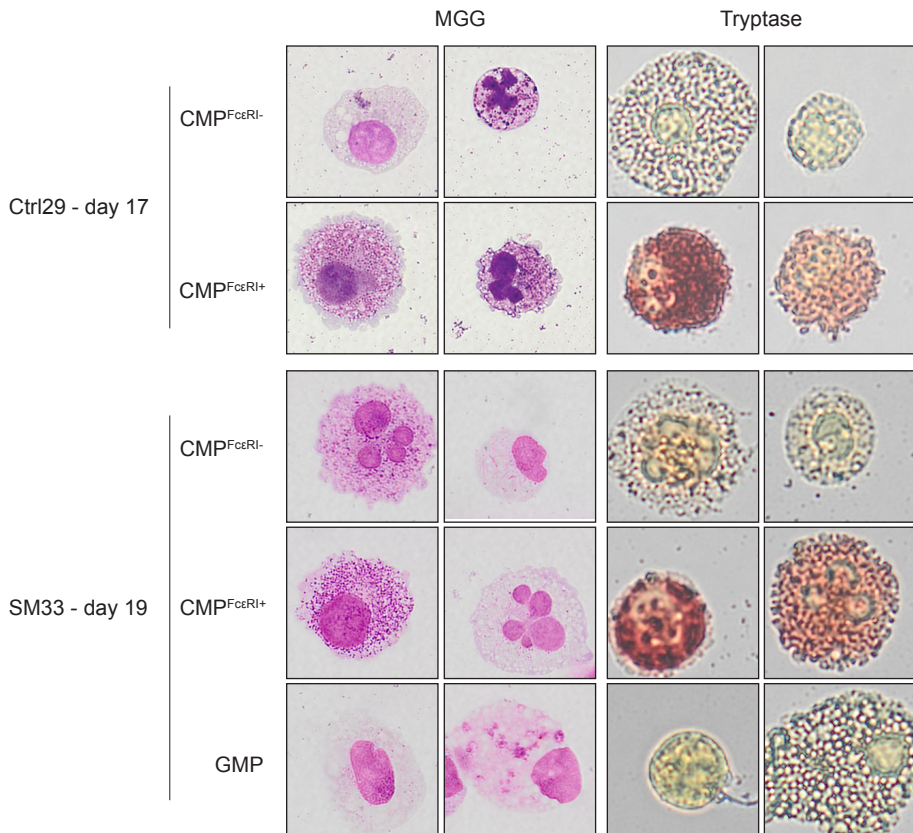
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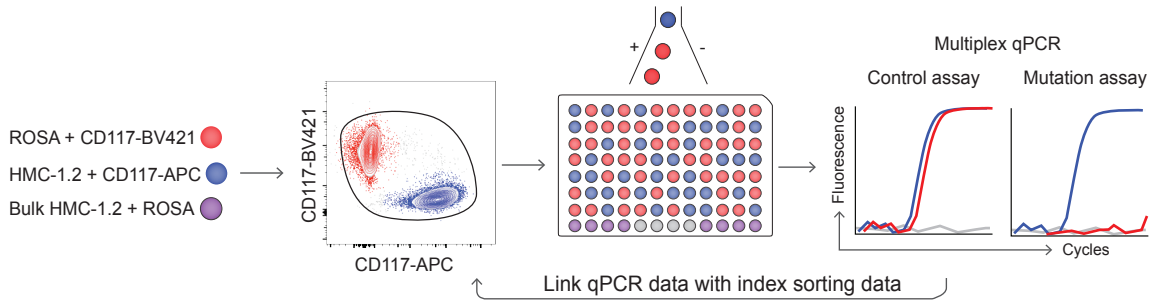


Supplementary Fig. 5

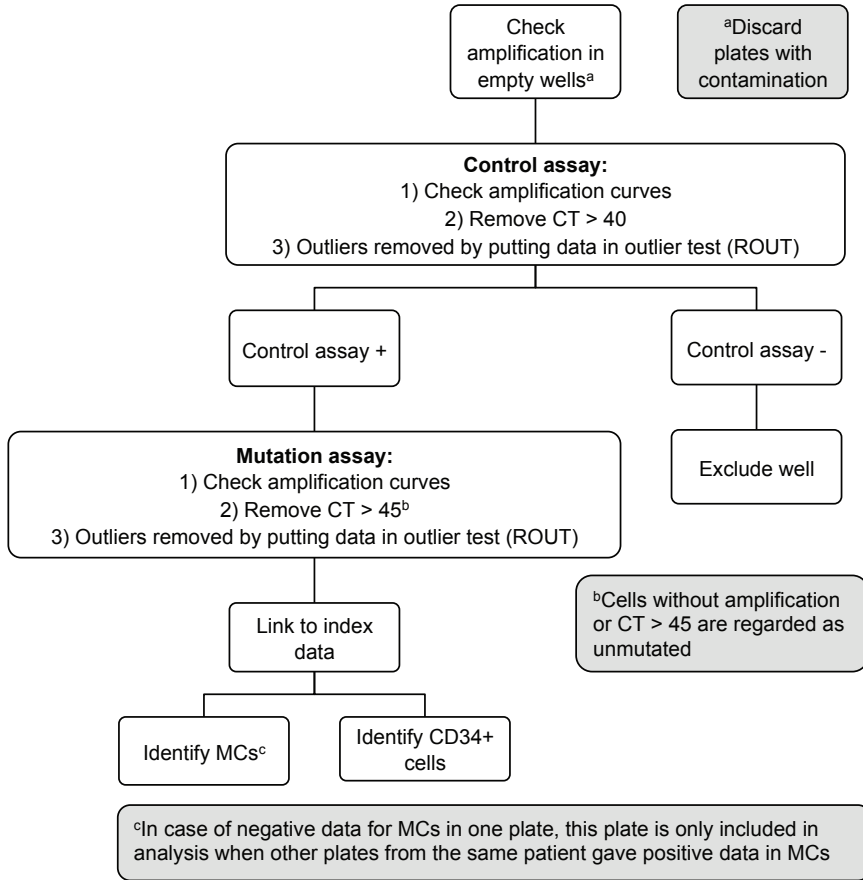


Supplementary Fig. 6

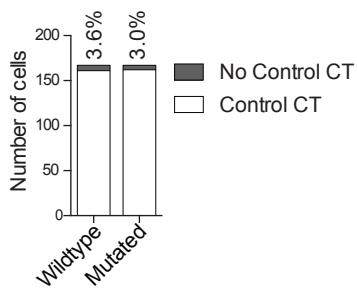
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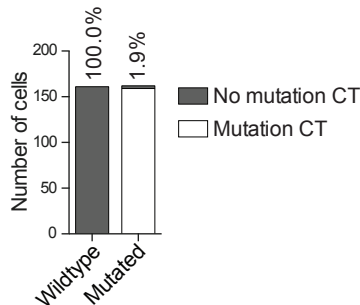
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c



d



Supplementary Fig. 7

