

Supplementary Figure 1: Localization of SMPX constructs in cultured cells

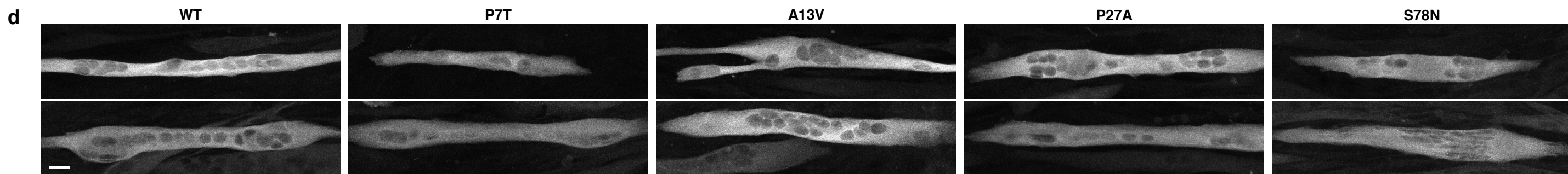
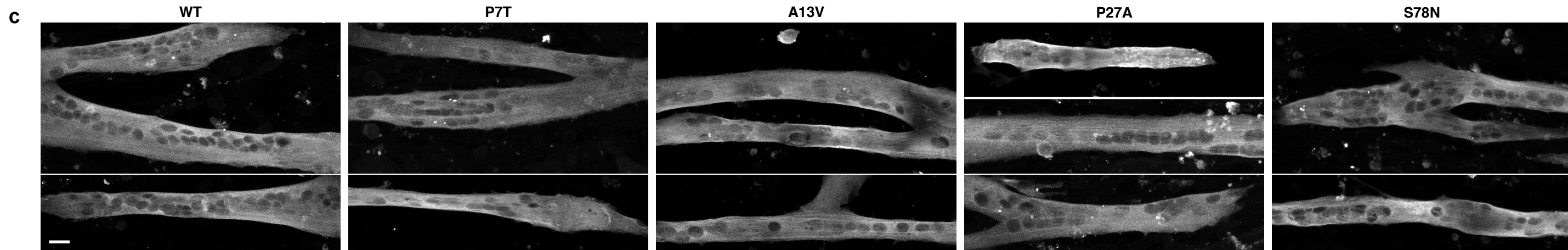
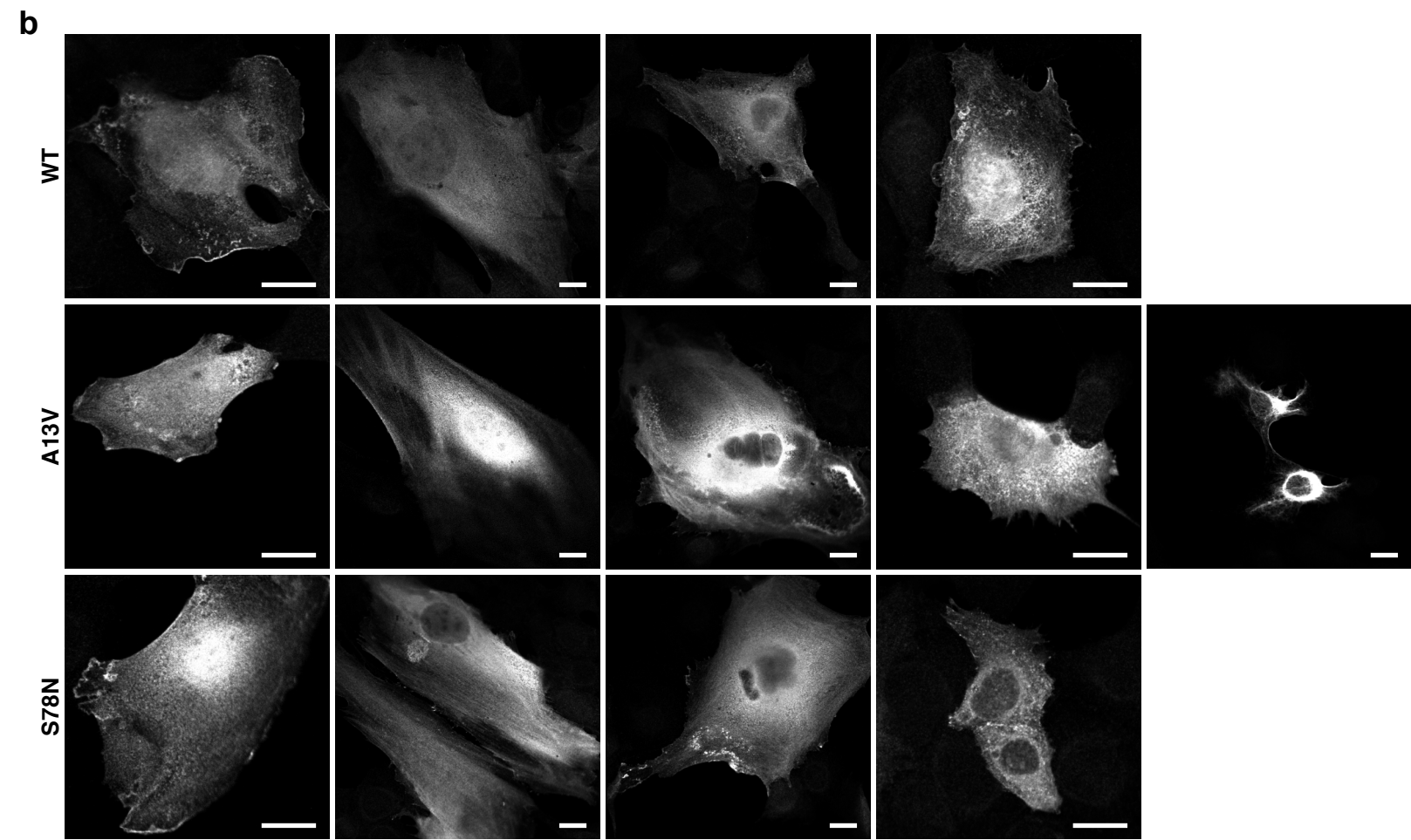
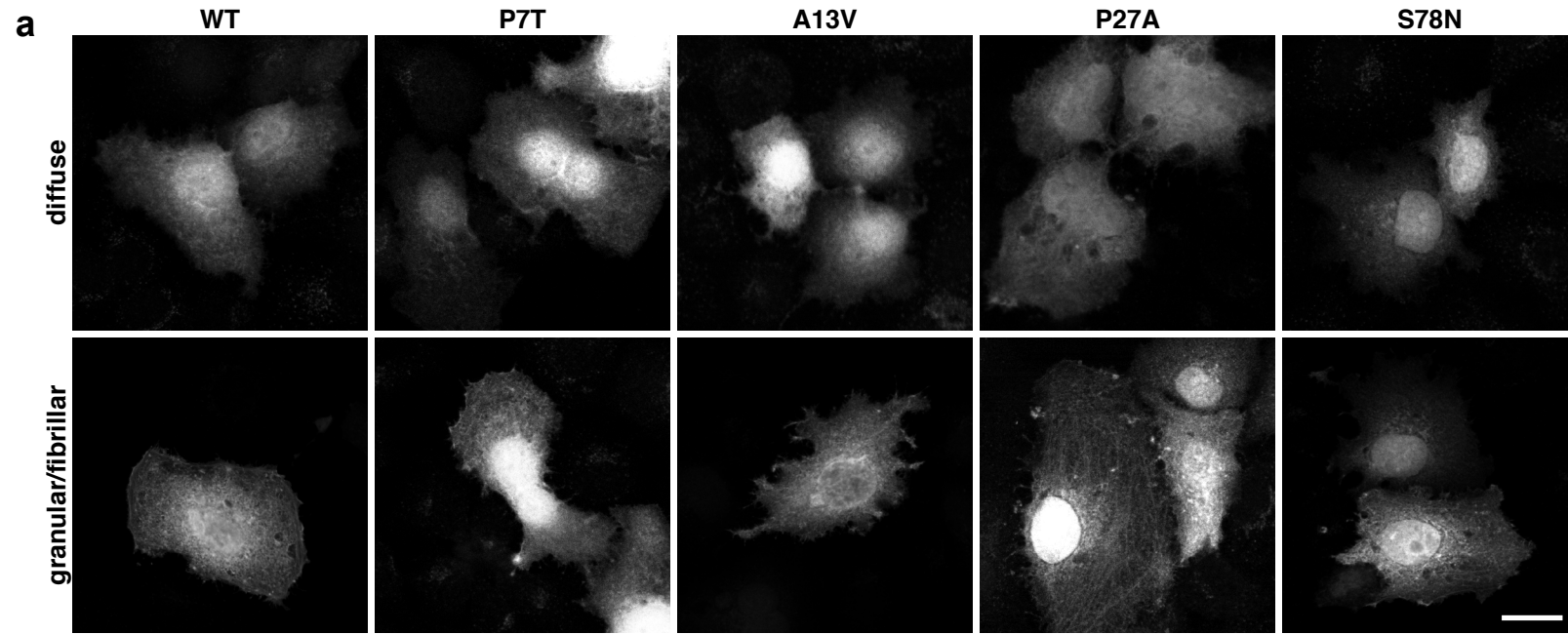
(a) SMPX-V5 in HeLa cells (single confocal sections)

(b) Myc-SMPX in C2C12 myoblasts (single confocal sections)

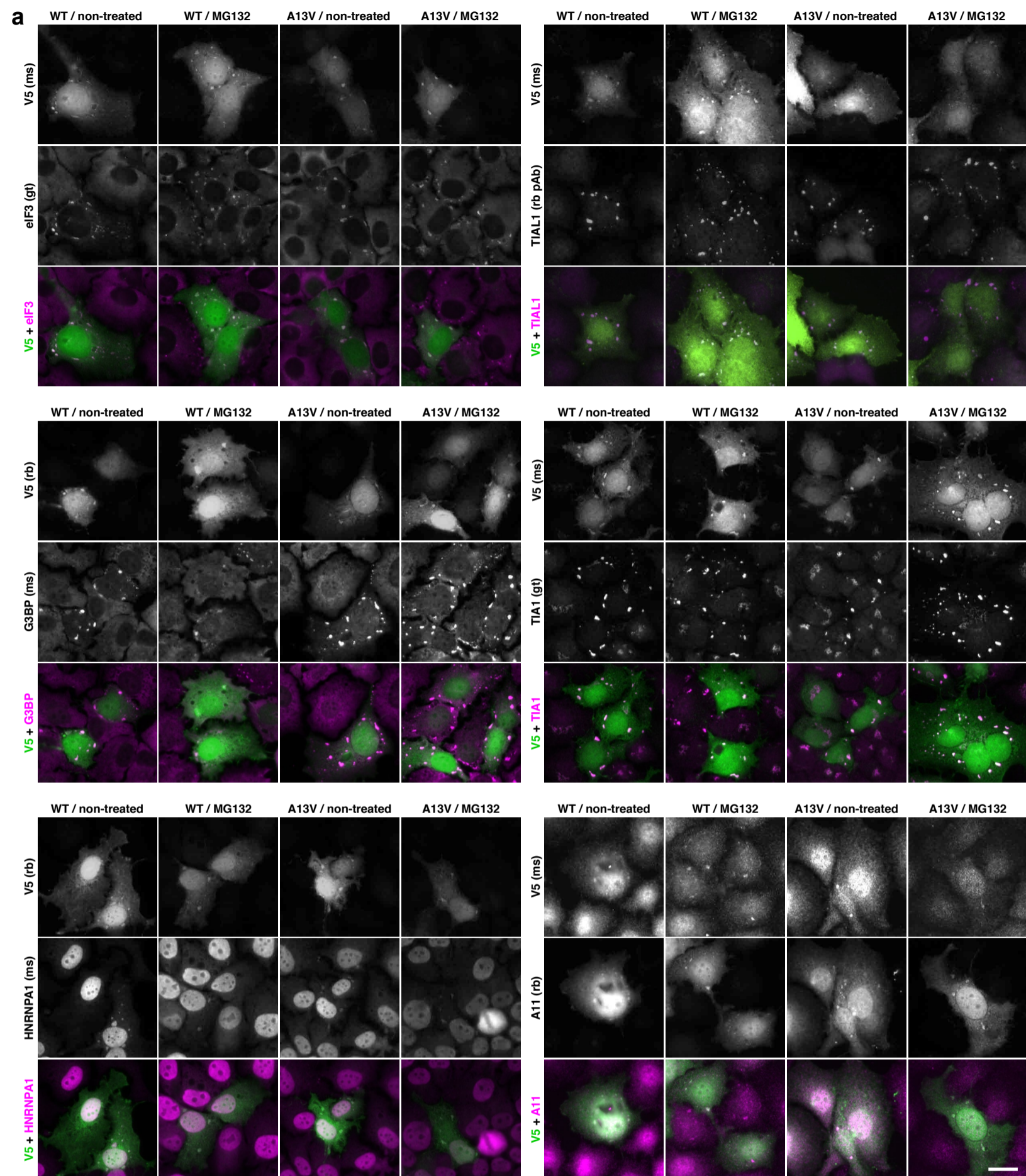
(c) SMPX-V5 in C2C12 myotubes (maximum intensity projections)

(d) Myc-SMPX in C2C12 myotubes (maximum intensity projections)

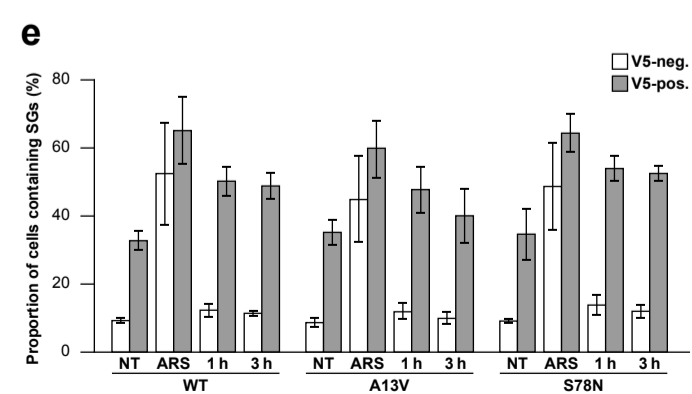
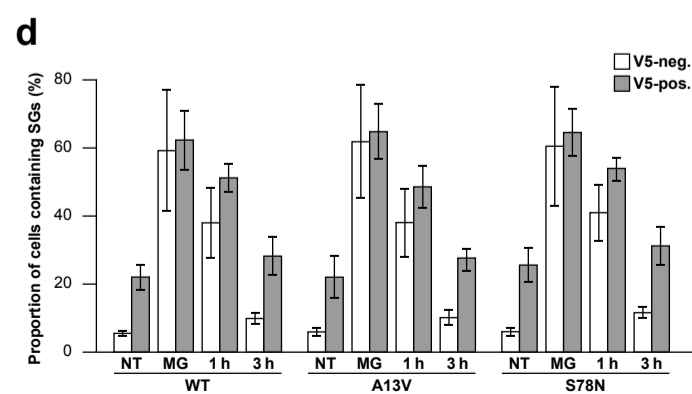
The image intensity levels have been individually adjusted and are not comparable between images. Scale bars = 20 μ m



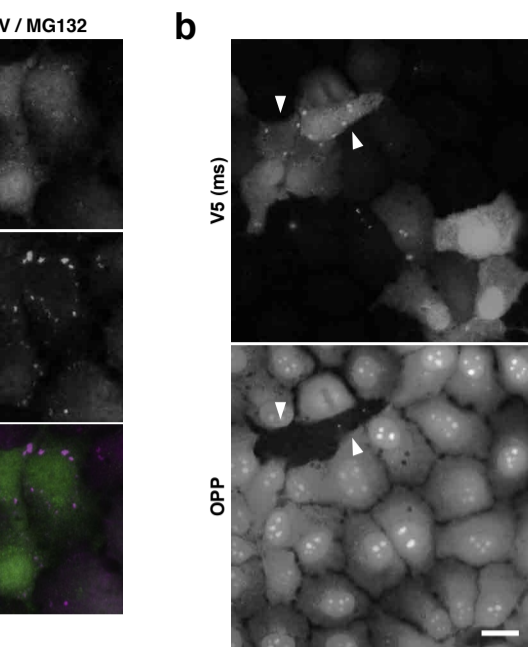
Supplementary Figure 2: SMPX and stress granules



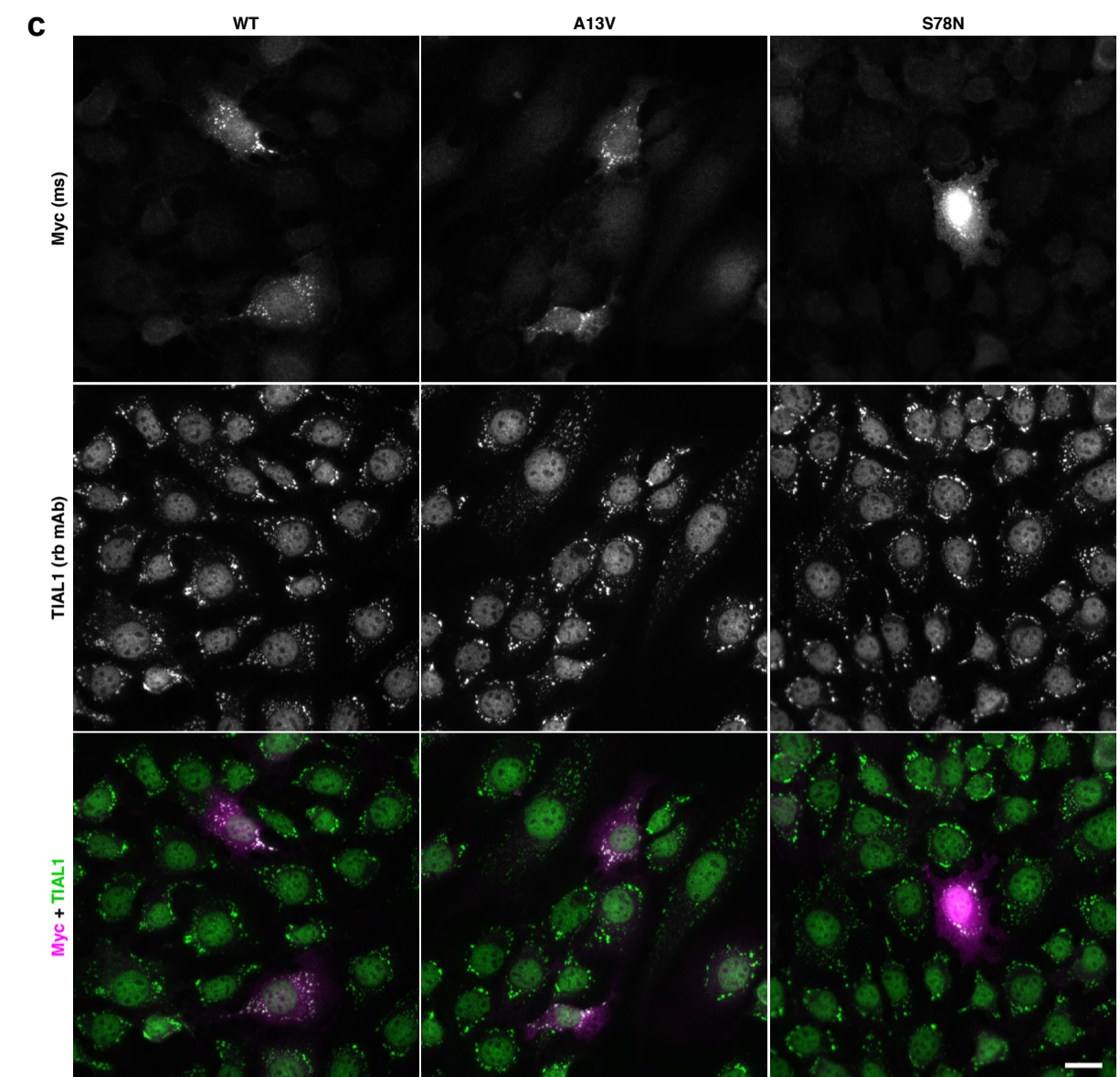
(a) Localization of SMPX-V5 to stress granules in HeLa cells. Wild-type (WT) and p.A13V SMPX-V5 were expressed in HeLa cells for 3 d. Cells were left non-treated or treated with 20 μ M MG132 for 2 h prior to fixing. SMPX-containing SGs stained positive for eIF3, G3BP, TIA1, TIAL1, HNRNPA1, and the amyloid oligomer antibody A11. Scale bar = 20 μ m.



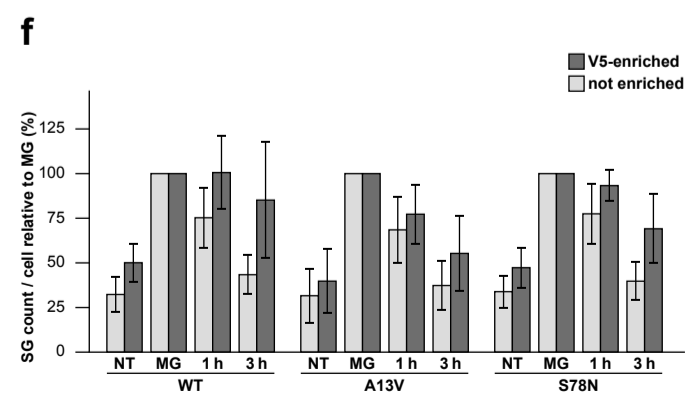
(d-e) The proportion of V5-negative and V5-positive cells containing ≥ 1 SGs in MG132 (d) and arsenite stress (e) experiments presented in Fig. 6b-c and Supplementary tables 2-3. The graphs show mean \pm SD of group means from 4 experiments.



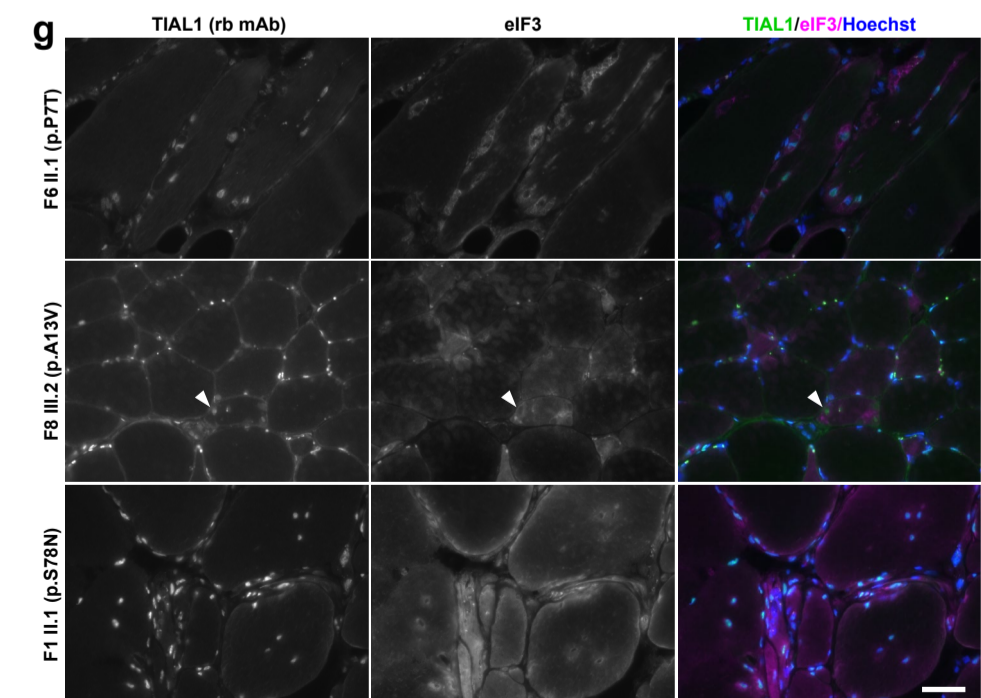
(b) Lack of OP-puromycin (OPP) incorporation indicating blocked protein synthesis in HeLa cells containing SMPX-V5 foci (arrowheads). Scale bar = 20 μ m.



(c) Localization of Myc-SMPX cells to stress granules in C2C12 myoblasts. Wild-type or mutant Myc-SMPX was expressed in stably transfected C2C12 myoblasts for 2 d, and SG formation was induced by heat shock (1 h in 45°C) before fixing. Scale bar = 20 μ m.



(f) Recovery of SMPX-V5-enriched and non-enriched SGs after MG132 stress. Data from Fig. 6d presented normalized to MG within each experiment. The graphs show mean \pm SD of group means from 4 experiments.



(g) IF analysis of SG components in patient muscle biopsies. In F6 II.1 and F8 III.2, accumulation of eIF3 was observed subsarcolemmally and surrounding the sarcoplasmic inclusions, a subset of which stained also positive for TIAL1 in F8 III.2 (arrowhead). In F1 II.1, an increased level of eIF3, but not TIAL1, was seen in the sarcoplasm of atrophic fibers. Scale bar = 50 μ m.

Supplementary Table 1: Immunohistochemical findings in SMPX patients

Antibody	protein inclusions	MFM
SMPX	+	-
p62	+	-
Ubiquitin	+	-
TDP-43	-	-
SMI-31	+	-
Myotilin	-	+
α B-crystallin	-	+

Supplementary Table 2: SG dynamics in MG132-stressed cells

SG dynamics (Fig. 7, Suppl. Fig. 2)			exp 1	exp 2	exp 3	exp 4	sum	mean	SD		
WT	NT	V5-neg. cells	cell count	5460	7189	7739	15415	35803			
			SGs/cell	0,060	0,077	0,094	0,105		0,084	0,020	
			cells with SGs	4,3%	5,4%	5,8%	6,6%		5,5%	1,0%	
		V5-pos. cells	cell count	866	922	986	1996	4770			
			SGs/cell	0,387	0,656	0,624	0,573		0,560	0,120	
			cells with SGs	16,3%	24,1%	24,2%	23,6%		22,1%	3,9%	
		MG	V5-neg. cells	cell count	6723	6336	13117	13023	39199		
			SGs/cell	1,683	1,884	0,752	0,768		1,272	0,596	
			cells with SGs	74,0%	75,6%	43,5%	43,9%		59,2%	18,0%	
		V5-pos. cells	cell count	1119	778	1624	1786	5307			
			SGs/cell	1,666	1,901	1,325	1,348		1,560	0,276	
			cells with SGs	69,3%	70,6%	54,3%	55,1%		62,3%	8,8%	
	R1	V5-neg. cells	cell count	5721	8996	12110	13673	40500			
		SGs/cell	0,870	0,595	0,396	0,625		0,622	0,194		
		cells with SGs	50,5%	37,6%	25,1%	38,8%		38,0%	10,4%		
	V5-pos. cells	cell count	1167	1408	1597	1987	6159				
		SGs/cell	1,136	1,281	1,165	1,339		1,230	0,095		
		cells with SGs	53,9%	52,3%	44,6%	54,0%		51,2%	4,4%		
	R3	V5-neg. cells	cell count	5001	10223	11856	11719	38799			
		SGs/cell	0,151	0,163	0,129	0,135		0,145	0,015		
		cells with SGs	11,5%	11,1%	7,8%	9,2%		9,9%	1,7%		
	V5-pos. cells	cell count	845	1439	1681	1613	5578				
		SGs/cell	0,400	0,915	0,814	0,893		0,755	0,241		
		cells with SGs	20,1%	32,3%	28,1%	32,3%		28,2%	5,7%		
A13V	NT	V5-neg. cells	cell count	5659	7075	10721	14137	37592			
			SGs/cell	0,056	0,081	0,105	0,124		0,092	0,030	
			cells with SGs	4,0%	5,6%	6,9%	7,3%		5,9%	1,5%	
		V5-pos. cells	cell count	1241	928	1555	1937	5661			
			SGs/cell	0,223	0,746	0,660	0,628		0,564	0,233	
			cells with SGs	12,7%	26,7%	23,7%	25,0%		22,0%	6,4%	
		MG	V5-neg. cells	cell count	6589	6522	14156	12578	39845		
			SGs/cell	1,793	1,973	0,836	0,886		1,372	0,595	
			cells with SGs	75,8%	76,8%	45,4%	49,3%		61,8%	16,8%	
		V5-pos. cells	cell count	1125	874	1794	1791	5584			
			SGs/cell	1,826	2,211	1,318	1,459		1,703	0,400	
			cells with SGs	70,7%	73,1%	56,4%	59,0%		64,8%	8,3%	
	R1	V5-neg. cells	cell count	5941	8592	13004	12526	40063			
		SGs/cell	0,797	0,570	0,357	0,748		0,618	0,200		
		cells with SGs	47,2%	37,0%	24,2%	44,0%		38,1%	10,2%		
	V5-pos. cells	cell count	1090	1317	1728	1825	5960				
		SGs/cell	1,154	1,160	0,962	1,369		1,161	0,166		
		cells with SGs	52,5%	48,0%	39,6%	54,2%		48,6%	6,5%		
	R3	V5-neg. cells	cell count	5320	9636	11614	12047	38617			
		SGs/cell	0,155	0,167	0,128	0,127		0,144	0,020		
		cells with SGs	12,3%	12,2%	7,6%	8,5%		10,2%	2,4%		
	V5-pos. cells	cell count	1096	1421	1499	1815	5831				
		SGs/cell	0,450	0,755	0,817	0,739		0,690	0,164		
		cells with SGs	22,9%	30,5%	28,8%	28,4%		27,7%	3,3%		
S78N	NT	V5-neg. cells	cell count	4985	4109	4183	13305	26582			
			SGs/cell	0,058	0,099	0,105	0,110		0,093	0,024	
			cells with SGs	4,1%	5,9%	7,1%	7,0%		6,0%	1,4%	
		V5-pos. cells	cell count	904	598	564	1832	3898			
			SGs/cell	0,423	0,895	0,707	0,663		0,672	0,194	
			cells with SGs	19,2%	31,8%	26,6%	24,7%		25,6%	5,2%	
		MG	V5-neg. cells	cell count	7455	8130	8912	8749	33246		
			SGs/cell	1,654	1,900	0,964	0,716		1,309	0,560	
			cells with SGs	73,3%	77,5%	50,0%	41,3%		60,5%	17,6%	
		V5-pos. cells	cell count	1343	1135	1110	1228	4816			
			SGs/cell	1,695	2,075	1,594	1,518		1,720	0,247	
			cells with SGs	68,1%	73,0%	58,9%	58,2%		64,6%	7,2%	
	R1	V5-neg. cells	cell count	7584	10373	12067	12624	42648			
		SGs/cell	0,892	0,633	0,495	0,734		0,689	0,167		
		cells with SGs	50,5%	39,7%	30,3%	43,4%		41,0%	8,4%		
	V5-pos. cells	cell count	1241	1518	1684	1892	6335				
		SGs/cell	1,315	1,418	1,324	1,559		1,404	0,113		
		cells with SGs	56,3%	54,2%	48,8%	56,7%		54,0%	3,6%		
	R3	V5-neg. cells	cell count	6994	7254	7896	12051	34195			
		SGs/cell	0,168	0,179	0,171	0,146		0,166	0,014		
		cells with SGs	13,0%	13,1%	10,8%	9,5%		11,6%	1,8%		
	V5-pos. cells	cell count	1176	1072	1146	1772	5166				
		SGs/cell	0,512	1,027	0,846	0,931		0,829	0,224		
		cells with SGs	24,1%	37,9%	30,1%	32,7%		31,2%	5,7%		

Supplementary Table 3: SG dynamics in arsenite-stressed cells

SG dynamics (Fig. 7, Suppl. Fig. 2)			exp 1	exp 2	exp 3	exp 4	sum	mean	SD	
WT	NT	cell count	5762	9723	4704	5913	26102			
		V5-neg. cells	SGs/cell	0,111	0,132	0,149	0,160		0,138	0,021
		cells with SGs	8,0%	9,4%	9,5%	10,3%		9,3%	0,9%	
	V5-pos. cells	cell count	707	757	538	561	2563			
		SGs/cell	0,622	0,871	1,097	1,021		0,903	0,209	
		cells with SGs	28,4%	32,9%	34,9%	34,8%		32,8%	3,0%	
	ARS	V5-neg. cells	cell count	14903	17468	12860	10240	55471		
		SGs/cell	1,434	1,802	0,662	0,838		1,184	0,528	
		cells with SGs	61,7%	68,7%	36,6%	42,9%		52,5%	15,1%	
	V5-pos. cells	cell count	1841	1593	1485	739	5658			
		SGs/cell	2,083	2,204	1,719	1,330		1,834	0,394	
		cells with SGs	71,8%	74,9%	60,6%	53,2%		65,1%	10,0%	
R1	V5-neg. cells	cell count	8753	14609	10501	13893	47756			
	SGs/cell	0,244	0,157	0,150	0,201		0,188	0,044		
	cells with SGs	15,2%	10,9%	10,5%	12,8%		12,3%	2,2%		
V5-pos. cells	cell count	656	1047	1030	1128	3861				
	SGs/cell	1,299	1,363	1,171	1,499		1,333	0,137		
	cells with SGs	55,2%	46,6%	46,5%	52,7%		50,2%	4,4%		
R3	V5-neg. cells	cell count	5404	13076	na	8034	26514			
	SGs/cell	0,220	0,157	na	0,188		0,188	0,032		
	cells with SGs	12,3%	10,5%	na	11,5%		11,4%	0,9%		
V5-pos. cells	cell count	498	1040	na	601	2139				
	SGs/cell	1,506	1,400	na	1,602		1,503	0,101		
	cells with SGs	52,6%	44,8%	na	49,1%		48,8%	3,9%		
A13V	NT	cell count	9730	9674	9499	8364	37267			
		V5-neg. cells	SGs/cell	0,097	0,136	0,167	0,125		0,131	0,029
		cells with SGs	7,2%	9,1%	10,6%	7,9%		8,7%	1,5%	
	V5-pos. cells	cell count	811	711	879	579	2980			
		SGs/cell	0,732	1,020	1,003	1,090		0,961	0,157	
		cells with SGs	29,5%	38,4%	35,8%	37,1%		35,2%	4,0%	
	ARS	V5-neg. cells	cell count	15268	13731	21500	11192	61691		
		SGs/cell	0,808	1,615	0,592	0,746		0,940	0,459	
		cells with SGs	42,4%	63,4%	34,3%	39,3%		44,9%	12,8%	
	V5-pos. cells	cell count	1216	1042	1562	549	4369			
		SGs/cell	1,433	2,194	1,249	1,508		1,596	0,413	
		cells with SGs	57,4%	72,5%	53,0%	56,8%		59,9%	8,6%	
R1	V5-neg. cells	cell count	9689	10044	13097	15698	48528			
	SGs/cell	0,243	0,137	0,170	0,180		0,182	0,044		
	cells with SGs	15,2%	9,2%	11,4%	11,7%		11,9%	2,5%		
V5-pos. cells	cell count	599	607	974	836	3016				
	SGs/cell	1,419	1,082	1,142	1,240		1,221	0,147		
	cells with SGs	58,1%	42,7%	45,5%	44,9%		47,8%	7,0%		
R3	V5-neg. cells	cell count	10624	8169	na	6669	25462			
	SGs/cell	0,176	0,108	na	0,150		0,145	0,034		
	cells with SGs	11,7%	7,9%	na	10,2%		9,9%	2,0%		
V5-pos. cells	cell count	801	508	na	401	1710				
	SGs/cell	1,449	1,122	na	1,202		1,258	0,171		
	cells with SGs	49,4%	36,6%	na	34,2%		40,1%	8,2%		
S78N	NT	cell count	8830	6207	6935	7198	29170			
		V5-neg. cells	SGs/cell	0,109	0,151	0,153	0,133		0,136	0,020
		cells with SGs	8,4%	10,0%	9,6%	8,5%		9,1%	0,8%	
	V5-pos. cells	cell count	1214	790	832	785	3621			
		SGs/cell	0,528	0,834	1,210	1,149		0,930	0,315	
		cells with SGs	24,7%	32,5%	42,2%	39,2%		34,7%	7,8%	
	ARS	V5-neg. cells	cell count	11305	15189	22526	12082	61102		
		SGs/cell	0,964	1,731	0,662	0,834		1,048	0,472	
		cells with SGs	48,9%	66,6%	36,6%	42,7%		48,7%	13,0%	
	V5-pos. cells	cell count	1425	1810	1556	941	5732			
		SGs/cell	1,587	2,277	1,638	1,775		1,819	0,316	
		cells with SGs	62,1%	72,9%	60,9%	61,4%		64,3%	5,7%	
R1	V5-neg. cells	cell count	14283	14956	12123	15193	56555			
	SGs/cell	0,322	0,187	0,219	0,184		0,228	0,065		
	cells with SGs	18,2%	11,5%	13,9%	11,8%		13,8%	3,1%		
V5-pos. cells	cell count	1190	1673	1356	1284	5503				
	SGs/cell	1,679	1,551	1,626	1,463		1,580	0,094		
	cells with SGs	59,1%	50,4%	54,8%	51,6%		54,0%	3,9%		
R3	V5-neg. cells	cell count	15787	11557	na	10653	37997			
	SGs/cell	0,236	0,189	na	0,152		0,192	0,042		
	cells with SGs	14,3%	11,5%	na	10,2%		12,0%	2,1%		
V5-pos. cells	cell count	1692	1232	na	916	3840				
	SGs/cell	1,680	1,824	na	1,892		1,798	0,108		
	cells with SGs	54,8%	50,2%	na	52,6%		52,5%	2,3%		

na, omitted due to failed staining

Supplementary Table 4: Effect of SMPX-V5 enrichment on SG recovery

V5 enrichment (Fig. 7, Suppl. Fig. 2)			exp 1	exp 2	exp 3	exp 4	mean	SD	
WT	NT	not V5-enr. SGs	per cell	0,308	0,556	0,542	0,462	0,467	0,114
		norm. to MG		18,8 %	30,2 %	43,0 %	37,3 %	32,3 %	10,4 %
	V5-enr. SGs	per cell	0,122	0,155	0,143	0,158	0,145	0,016	
		norm. to MG		41,1 %	40,5 %	63,3 %	55,4 %	50,1 %	11,2 %
	MG	not V5-enr. SGs	per cell	1,641	1,841	1,259	1,241	1,495	0,295
		norm. to MG		100,0 %	100,0 %	100,0 %	100,0 %	100,0 %	
	V5-enr. SGs	per cell	0,298	0,383	0,226	0,286	0,298	0,065	
		norm. to MG		100,0 %	100,0 %	100,0 %	100,0 %	100,0 %	
	R1	not V5-enr. SGs	per cell	1,072	1,077	1,015	1,200	1,091	0,078
		norm. to MG		65,3 %	58,5 %	80,6 %	96,7 %	75,3 %	17,0 %
	V5-enr. SGs	per cell	0,237	0,347	0,291	0,296	0,293	0,045	
		norm. to MG		79,5 %	90,5 %	128,6 %	103,8 %	100,6 %	21,1 %
R3	not V5-enr. SGs	per cell	0,491	0,716	0,624	0,684	0,629	0,099	
	norm. to MG		29,9 %	38,9 %	49,6 %	55,1 %	43,4 %	11,2 %	
V5-enr. SGs	per cell	0,133	0,283	0,268	0,296	0,245	0,076		
	norm. to MG		44,5 %	73,8 %	118,7 %	103,6 %	85,2 %	32,9 %	
A13V	NT	not V5-enr. SGs	per cell	0,187	0,647	0,565	0,498	0,474	0,201
		norm. to MG		10,4 %	31,3 %	47,0 %	37,8 %	31,6 %	15,6 %
	V5-enr. SGs	per cell	0,047	0,165	0,167	0,171	0,137	0,060	
		norm. to MG		14,6 %	37,5 %	54,4 %	52,5 %	39,8 %	18,4 %
	MG	not V5-enr. SGs	per cell	1,805	2,066	1,200	1,318	1,597	0,408
		norm. to MG		100,0 %	100,0 %	100,0 %	100,0 %	100,0 %	
	V5-enr. SGs	per cell	0,319	0,439	0,306	0,326	0,348	0,062	
		norm. to MG		100,0 %	100,0 %	100,0 %	100,0 %	100,0 %	
	R1	not V5-enr. SGs	per cell	1,068	1,034	0,857	1,231	1,048	0,153
		norm. to MG		59,2 %	50,0 %	71,4 %	93,4 %	68,5 %	18,8 %
	V5-enr. SGs	per cell	0,224	0,281	0,223	0,333	0,265	0,053	
		norm. to MG		70,1 %	63,9 %	72,8 %	102,2 %	77,3 %	17,0 %
R3	not V5-enr. SGs	per cell	0,396	0,610	0,631	0,597	0,558	0,109	
	norm. to MG		21,9 %	29,5 %	52,6 %	45,3 %	37,3 %	14,1 %	
V5-enr. SGs	per cell	0,095	0,212	0,241	0,210	0,190	0,065		
	norm. to MG		29,7 %	48,2 %	78,7 %	64,7 %	55,3 %	21,1 %	
S78N	NT	not V5-enr. SGs	per cell	0,346	0,689	0,601	0,507	0,536	0,147
		norm. to MG		20,5 %	34,9 %	42,1 %	38,2 %	33,9 %	9,4 %
	V5-enr. SGs	per cell	0,107	0,264	0,163	0,216	0,188	0,068	
		norm. to MG		33,1 %	56,5 %	43,0 %	56,7 %	47,3 %	11,5 %
	MG	not V5-enr. SGs	per cell	1,692	1,974	1,429	1,329	1,606	0,289
		norm. to MG		100,0 %	100,0 %	100,0 %	100,0 %	100,0 %	
	V5-enr. SGs	per cell	0,325	0,468	0,379	0,380	0,388	0,059	
		norm. to MG		100,0 %	100,0 %	100,0 %	100,0 %	100,0 %	
	R1	not V5-enr. SGs	per cell	1,212	1,215	1,067	1,358	1,213	0,119
		norm. to MG		71,6 %	61,5 %	74,6 %	102,2 %	77,5 %	17,4 %
	V5-enr. SGs	per cell	0,287	0,388	0,388	0,378	0,360	0,049	
		norm. to MG		88,4 %	82,9 %	102,2 %	99,5 %	93,3 %	9,1 %
R3	not V5-enr. SGs	per cell	0,415	0,802	0,618	0,673	0,627	0,161	
	norm. to MG		24,5 %	40,6 %	43,2 %	50,6 %	39,8 %	11,0 %	
V5-enr. SGs	per cell	0,138	0,310	0,309	0,328	0,271	0,089		
	norm. to MG		42,4 %	66,2 %	81,4 %	86,4 %	69,1 %	19,7 %	