Supplemental Data

Methods

TUNEL staining

The TUNEL cell death assay was carried out using the "In Situ Cell Death Detection Kit" TMR red (Roche, Indianapolis, IN, USA) according to manufacturer's instructions.

In vitro tube formation

HUVEC were maintained in EBM-2 medium supplemented with EGM-2. Tube formation assay on extracellular BD matrigel was performed according to the manufacturer's protocol with minor modifications. 2×10^4 of HUVECs were resuspended with 500 µl of EBM-2 medium and were seeded on Matrixgel solidified in 48-wel tissue culture plate. Cells were incubated in a CO_2 incubator for 24 hrs at 37°C and then examined for tube formation with a light microscope.

RT-PCR

For RT-PCR, total mRNA was isolated from wild-type zebrafish embryos at several stages (24, 36, 48, and 72 hpf). Two specific forward primers that correspond to the N-terminal and a reverse primer to the C-terminal of zebrafish akap12 isoforms were used. Following primers were used in this study: akap12 α forward primer (ATGGGAGCGACACCATCCG), akap12 β forward primer (ATGCTTGGGACAATAACTCTAACAG), akap12 reverse primer (TGAGGCCTGTCTTCTGTTGA), β-actin forward primer (GATCTTCACTCCCCTTGTTCAC) and β-actin reverse primer (CACAGCTTCTCCT TGATGTCAC).

In situ hybridization (ISH)

For ISH, wild-type zebrafish embryos at several stages (24, 48, and 72 hpf) were used. Due to the small size of the specific sequence for each akap12 isoform (akap12α, 212 nt; akap12β, 23 nt), we designed a pan-akap12 probe. To generate the *akap12* riboprobe, 2256 base pairs of the conserved region of akap12α and -β were cloned into the pGEM-T Easy vector (Promega, WI, USA). A DIG-labeled akap12 antisense riboprobe was synthesized using a DIG RNA labeling

kit (Roche, IN, USA). Standard ISH was performed as described (Oxtoby and Jowett, 1993; Strähle et al., 1994).

Translation blocking Morpholino (MO) specificity test

Zebrafish akap12 isoforms, akap12α and akap12β, are cloned into pEGFP-N3 vector (Clontech, CA, USA) and amplified by PCR using specific primer containing SP6 promoter sequence for *in vitro* transcription. *In vitro* transcribed mRNAs (*akap12α-gfp,* 25pg; *akap12β-gfp*, 25pg) and translation blocking MOs (akap12α, 2ng; akap12β, 7.5ng) are injected into zebrafish embryos at 1 to 4-cell stage and then acquired the fluorescence image at 24hpf. Following primers were used in this study: forward primer (SP6) (TTGATTTAGGTGACACTATAGAGCGCTACCG GACTCAGATCTC), reverse primer (TTACTTGTACAGCTCGRCCATGC).

Figure Legends

Supplemental Figure 1. Construction of morpholino and probe for akap12

A-B, The genetic map of zebrafish akap12α and β (A). The arched lines connecting the exons represent the splicing of the two *akap12* mRNAs. The *akap12α* includes exons 1, 2 and 4. The *akap12β* includes exons 3 and 4. Target regions of MOs were indicated by green bar for splicing blocker (MO1), by red bar for translation blocker (MO2), respectively. *Akap12* mRNA probe for ISH was designed on the basis of akap12-genetic map (blue bar). RT-PCR analyses for *akap12* isoform (B). The efficiency of MO1 for akap12 was examined by using specific primer for each akap12 isoform (green arrow) (A). C, The efficacy of akap12 MO2 was examined by the co-injection of in vitro transcribed *akap12-gfp* mRNA (*akap12α-gfp*, 25pg; *akap12β-gfp,* 25pg) and akap12 MO2 (α MO2, 2ng; βMO2, 7.5ng). Note that the expression of GFP was blocked by injecting akap12 MO2. D-E, Lateral view of akap12 morphants with normal trunk (D) and with trunk defect (E). Hemorrhages were indicated by red arrows. αF, akap12α forward primer; βF, akap12β forward primer; αβR, reverse primer for akap12α and akap12β, MO1, splicing blocking MO; MO2, translation blocking MO; N, normal trunk; TD, trunk defect.

Supplemental Figure 2. Hemorrhages are not caused by vessel regression

Confocal images of the TUNEL stained Tg(fli1a:EGFP)y1 zebrafish embryos (Ctl MO, 7.5ng; α MO1, 2ng; βMO1, 7.5ng) at 48 hpf. TUNEL positive signals were indicated by red dots. White boxed regions with numbers were enlarged. Yellow boxed regions were projected with different angles (2'R, 286°; 4'R, 107°; 5'R, 101°; 6'R, 101°) using Zeiss LSM program. Note that there were no differences in TUNEL positive signals between control and akap12 morphants. Scale bar, 100um. R, rotation.

Supplemental Figure 3. PAK2 and AF6-depleted HUVECs show the failure in tube formation

A, Immunoblots. HUVECs were treated with 10nmol/L siRNA for AKAP12 and immunostained with anti-AKAP12. B and C, *in vitro* tube formation assay. Note that capillary tubes in PAK2 and AF6-depleted HUVECs were broken. D and E, RT-PCR analysis using akap12 morphant at 48hpf (D) and AKAP12 depleted HUVECs after 48hours after siRNA transfection (E).

Supplementary table legend

Supplemental table 1. Statistics of hemorrhages of akap12 morphant

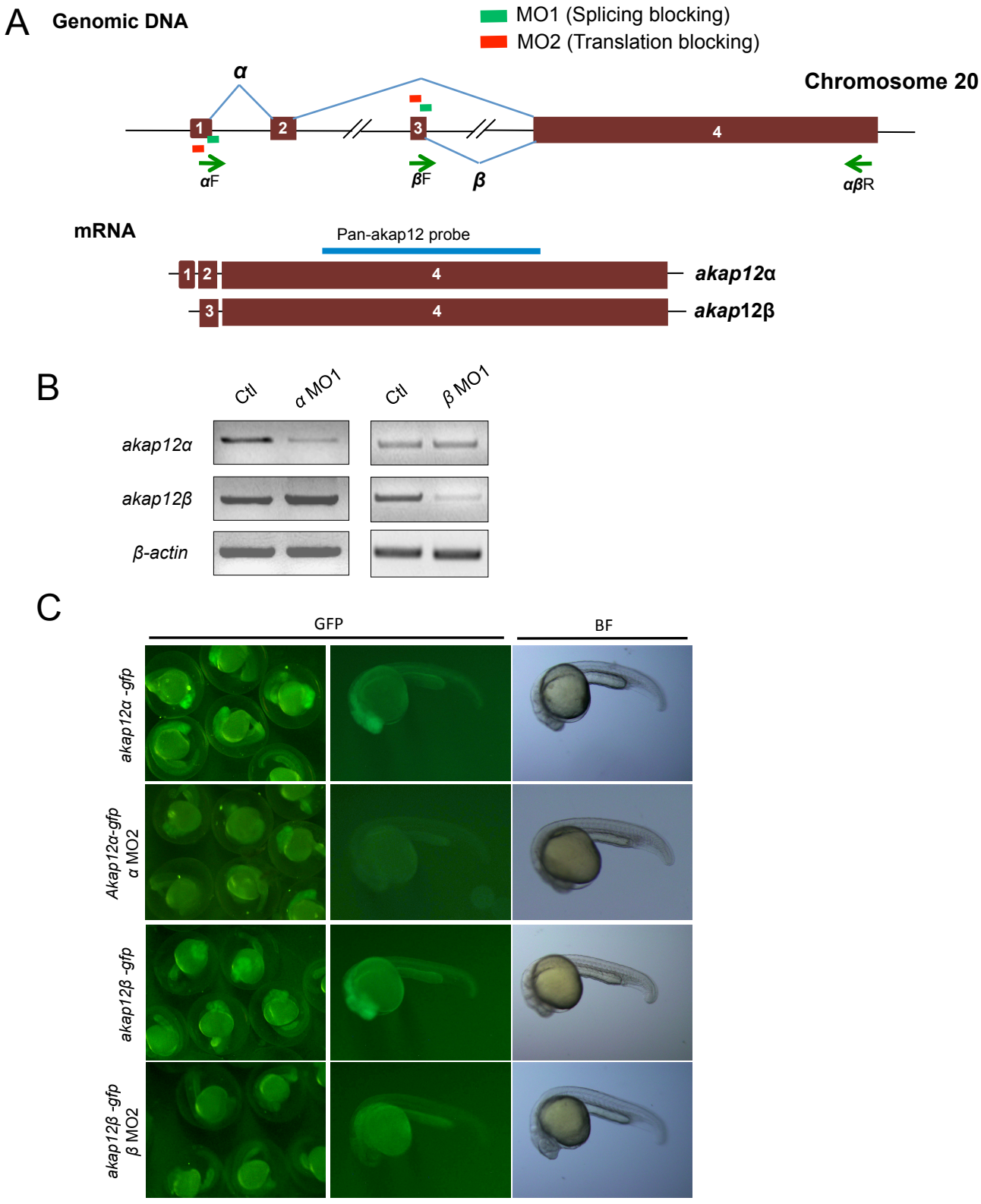
Incidence of hemorrhages was examined at 48-72hpf. Note that number in parenthesis indicates

embryos with trunk defects.

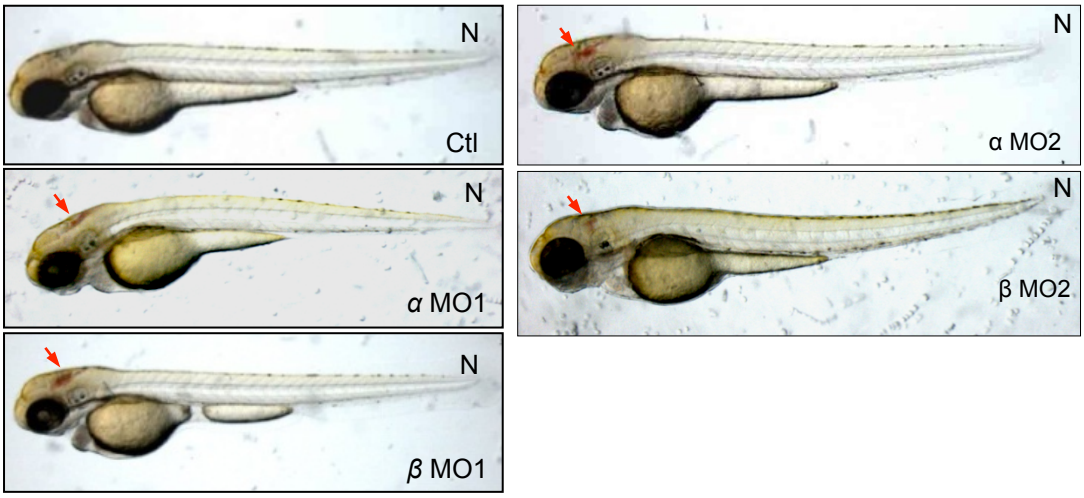
Supplemental table 2. Statistics of *akap12* mRNA rescue experiment
Incidence of hemorrhages was examined at 48-72hpf. Note that number in parenthesis indicates the amount of injected *akap12* mRNA.

Supplemental table 3. Statistics of *PAK2* and *AF6* mRNA rescue experiment
Incidence of hemorrhages was examined at 48-72hpf. Note that number in parenthesis indicates the amount of injected MOs.

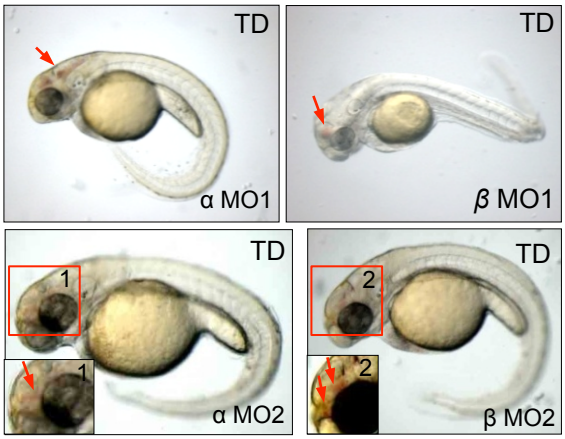
Supplemental Figure 1.



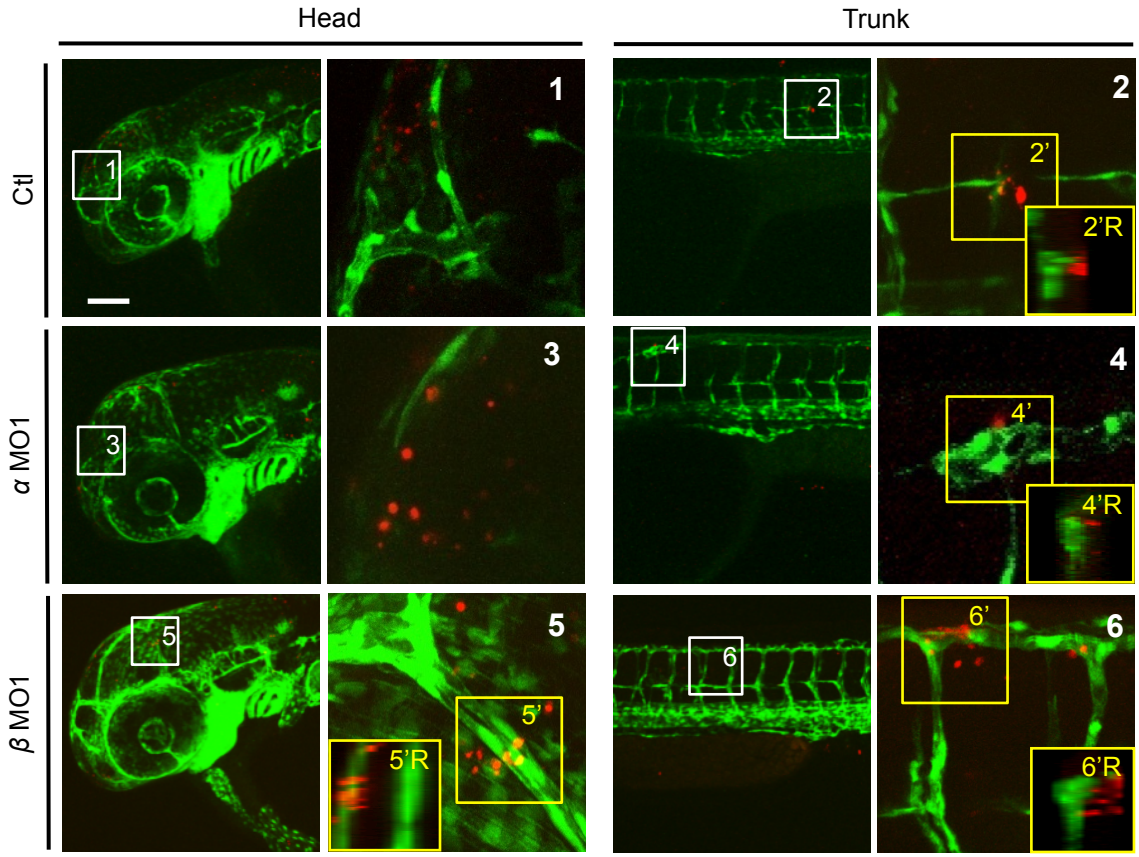
D


N
Ctl
N
α MO2
N
α MO1
N
β MO2
N
β MO1

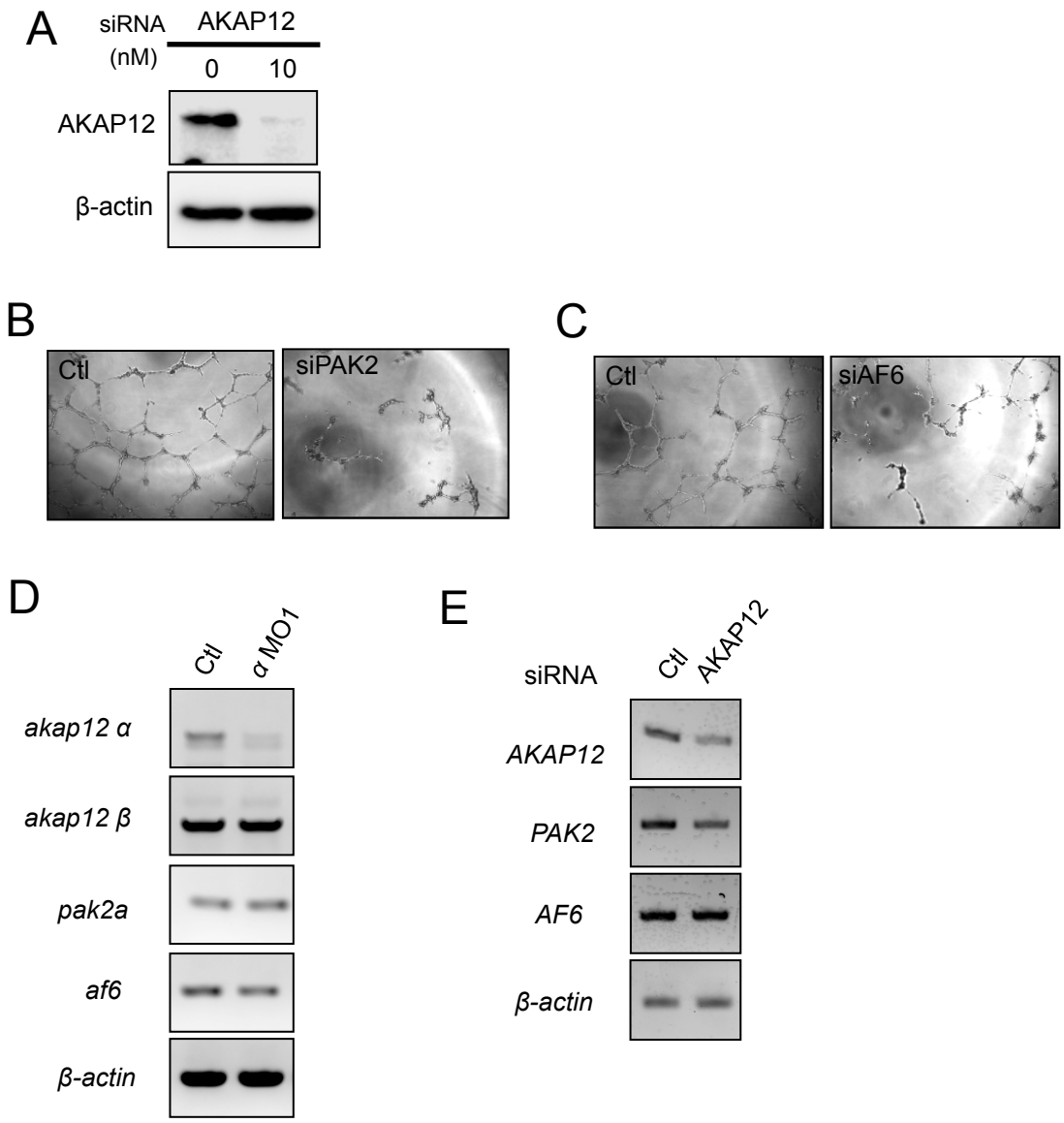
E


TD
α MO1
TD
β MO1
TD
1
1
α MO2
TD
2
2
β MO2

Supplemntal Figure 2.



Supplemental Figure 3.



Supplemental Table 1.

MO	ng	Number of embryos				Percentage (%)		
		Hemo	Non-hemo	Dead	Total	Hemo	Non-hemo	Dead
Ctl	0	0 (0)	96 (0)	2	98	0 (0)	98.0 (0)	2.0
	13	0 (0)	75 (0)	1	76	0 (0)	98.7 (0)	1.3
α MO1	0.6	28 (6)	90 (32)	1	119	23.5 (5.0)	75.6 (26.9)	0.8
	2	46 (28)	56 (16)	3	105	43.8 (26.7)	53.3 (15.2)	2.9
	3	1 (1)	64 (58)	7	72	1.4 (1.4)	88.9 (80.6)	9.7
β MO1	4.5	14 (4)	70 (28)	2	86	16.3 (4.7)	81.4 (32.6)	2.3
	9	11 (5)	52 (27)	8	71	15.5 (7.0)	73.2 (38.0)	11.3
	13	6 (2)	36 (28)	12	54	11.1 (3.7)	66.7 (51.9)	22.2

( ), Trunk defect embryos

Supplemental Table 2.

MO (ng)	**mRNA** (pg)	**Number of embryos**				**Percentage (%)**		
		Hemo	**Non-hemo**	**Dead**	**Total**	**Hemo**	**Non-hemo**	**Dead**
Ctl (7.5)	None (0)	0	132	1	133	0	99.2	0.8
	akap12α (50)	1	86	2	89	1.1	96.6	2.2
	akap12β (50)	0	93	3	96	0	96.9	3.1
α MO1 (2)	None (0)	51	56	3	110	46.4	50.9	2.7
	akap12α (50)	7	83	3	93	7.5	89.2	3.2
β MO1 (7.5)	None (0)	21	72	5	98	21.4	73.5	5.1
	akap12β (50)	8	64	3	75	10.7	85.3	4

Supplemental Table 3.

MO (ng)	mRNA*	Number of embryos				Percentage (%)		
		Hemo	Non-hemo	Dead	Total	Hemo	Non-hemo	Dead
Ctl (0)	*None*	0	83	0	83	0.0	100	0
	PAK2	0	59	1	60	0.0	98.3	1.7
	AF6	0	73	1	74	0.0	98.6	1.4
	PAK2 + AF6	0	70	0	70	0.0	100	0
α MO1 (2)	*None*	15	33	1	49	30.6	67.3	2.0
	PAK2	4	56	1	61	6.6	91.8	1.6
	AF6	1	75	3	79	1.3	94.9	3.8
	PAK2+ AF6	7	89	2	98	7.1	90.8	2.0
β MO1 (7.5)	*None*	6	39	2	47	12.8	83.0	4.3
	PAK2	1	54	3	58	1.7	93.1	5.2
	AF6	3	74	4	81	3.7	91.4	4.9
	PAK2+ AF6	3	78	2	83	3.6	94.0	2.4

* *PAK2, 25 pg; AF6, 25 pg*