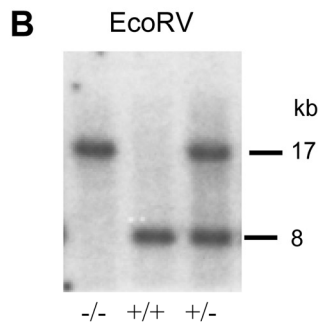
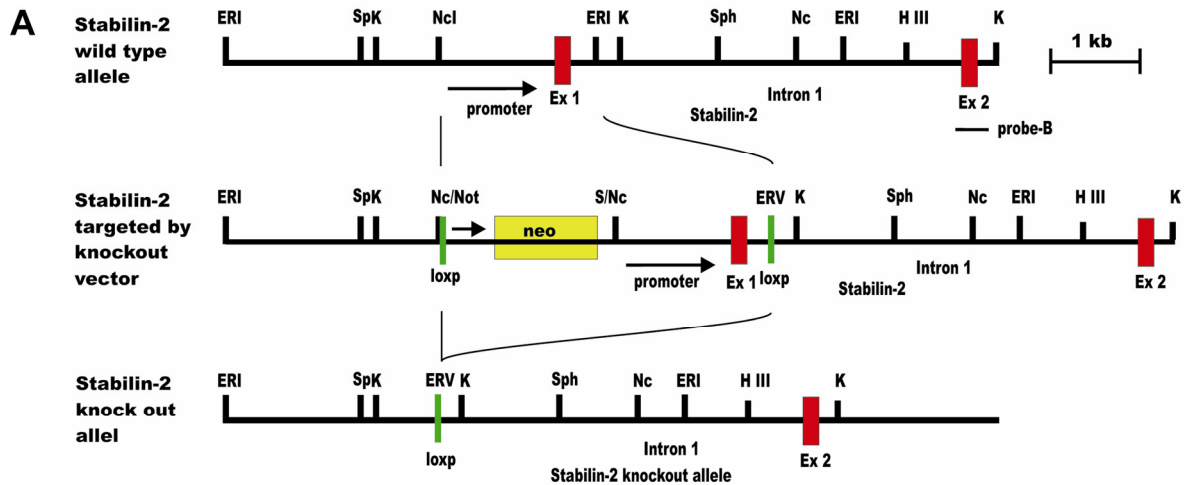


## Supplementary Figure 1

### Targeting strategy and generation of Stabilin-1 knockout allele.

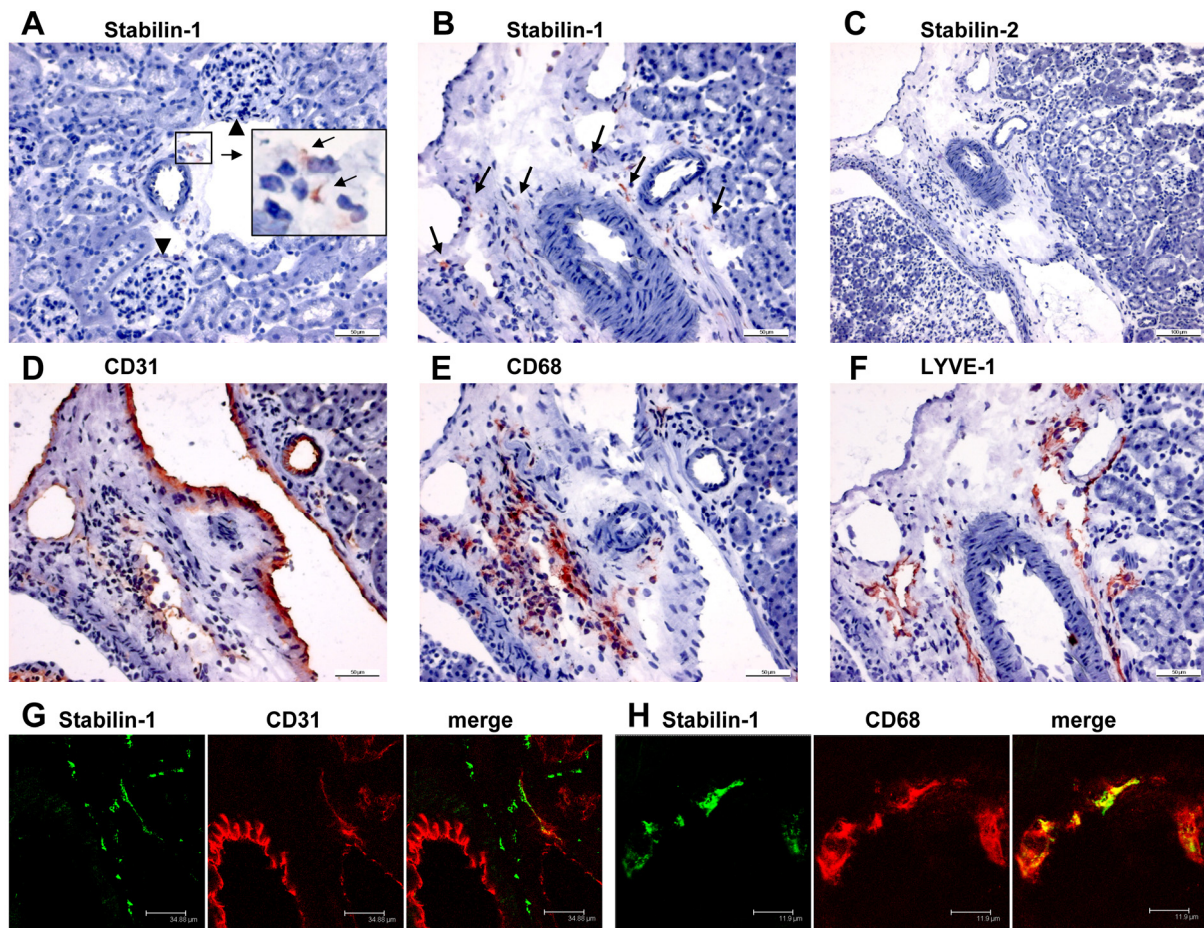
(A) Structural organization of the Stabilin-1 wild-type, knockout vector targeted, and Stabilin-1 knockout allele. The 2 *loxP* sites flanking the Stabilin-1 exon 1 (red) are shown in green bars. The relative positions of probe-A used for Southern blot analysis and restriction endonuclease sites relevant for cloning procedures indicated as K (*KpnI*), Sda I (*SdaI*), ERI (*EcoRI*), Xho (*XhoI*), S (*SmaI*), Not (*NotI*) are indicated. Cre-mediated deletion of Stabilin-1 exon 1 was achieved by crossing heterozygous *Stab-1* *+/-* mice with C57BL/6 cre-deleter expressing constitutively recombinase cre. F2 mice were screened for Stabilin-1 *+/-* mice. First Stabilin-1 *+/-* mice of mixed 129-C57BL/6 genetic background have been continuously backcrossed to either C57BL/6 or Balb/c mouse strains. (B) Southern blot analysis (according to standard procedures) of *EcoRI*-digested genomic DNA using probe-A clearly discriminates Stabilin-1 WT, knockout, and heterozygous genotypes.



## Supplementary Figure 2

### Targeting strategy and generation of Stablin-2 knockout allele.

(A) Structural organization of the Stablin-2 wild-type, knockout vector targeted, and Stablin-2 knockout allele. The 2 loxP sites flanking the Stablin-2 exon 1 (red) are shown in green bars. The relative positions of probe-B used for Southern blot analysis and restriction endonuclease sites relevant for cloning procedures indicated as ERI (*EcoRI*), Sp (*SpeI*) K (*KpnI*), Nc (*NcoI*), Sph (*SphI*), H III (*HindIII*), S (*SmaI*), Xho (*XhoI*) are indicated. Cre-mediated deletion of Stablin-2 exon 1 was achieved by crossing heterozygous Stab-2 +/flox mice with C57BL/6 cre-deleter expressing constitutively recombinase cre. F2 mice were screened for Stablin-2 +/- mice. First Stablin-2 +/- mice of mixed 129-C57BL/6 genetic background have been continuously backcrossed to either C57BL/6 or Balb/c mouse strains. (B) Southern blot analysis (according to standard procedures) of *EcoRV*-digested genomic DNA using probe-B clearly discriminates Stablin-2 WT, knockout, and heterozygous genotypes.

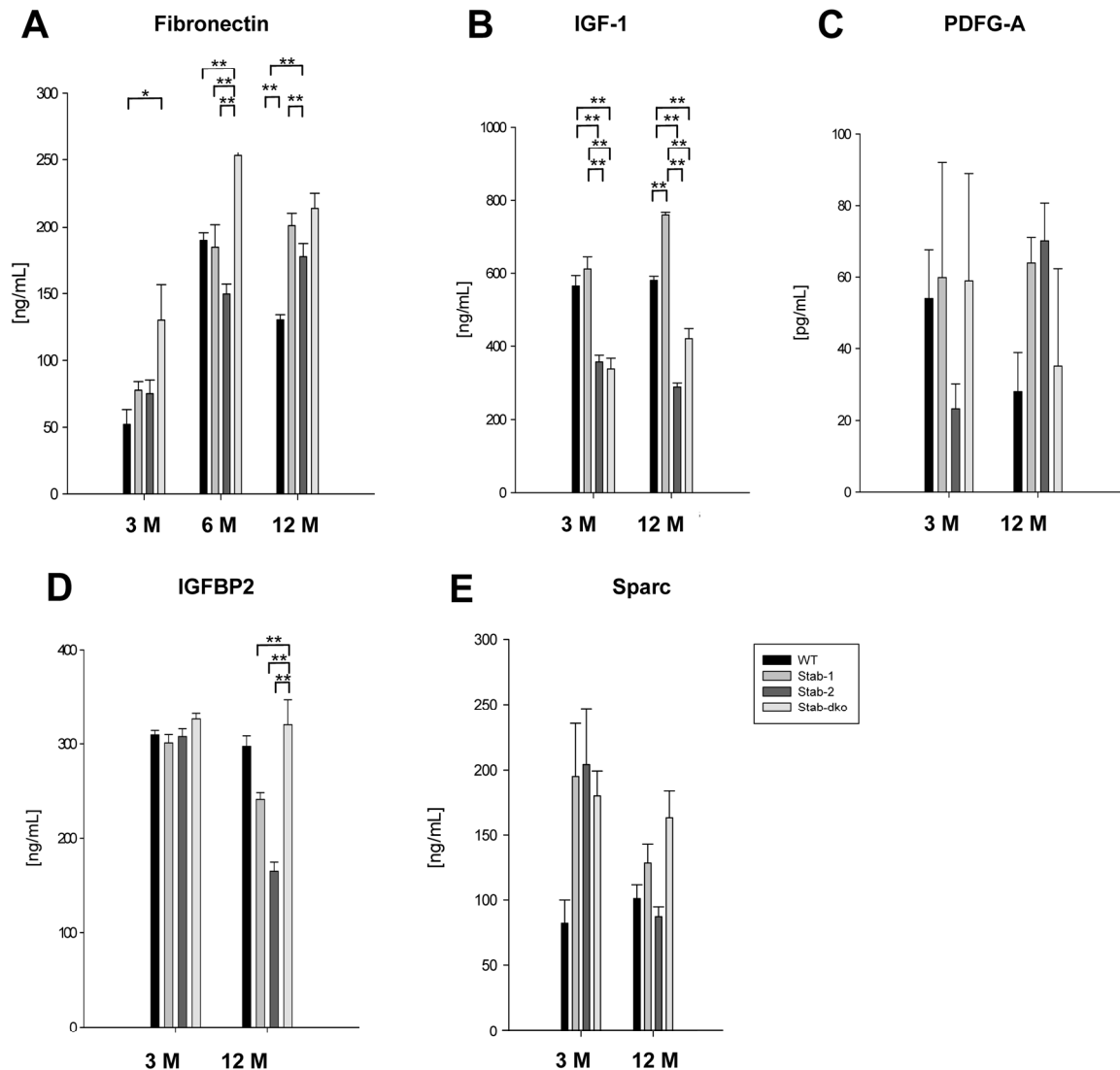


### Supplementary Figure 3

#### Stabilin-1 and Stabilin-2 expression in mouse kidney.

(A-F) Representative kidney sections of 2 month old C57BL/6 WT were stained by immunohistochemistry with antibodies against (A, B) Stabilin-1, (C) Stabilin-2, (D) vessel marker CD31 (serotec), (E) macrophage marker CD68 (Acris) and (F) lymph vessel marker LYVE-1 (Abcam). (A) Only few stabilin-1 positive single cells were found in mouse kidney and most of them were located perivascularly (boxed frame, arrows). No Stabilin-1 expression was detected in glomerular structures (arrowheads). (B) Close to larger vessels we observed a higher incidence of Stabilin-1 expressing single cells (arrows). (A, B) Original magnification 200x. Scale bar 50  $\mu\text{m}$ . (C) In contrast, no Stabilin-2 positive cells have been detected in mouse kidney. (Original magnification 100x. Scale bar 100  $\mu\text{m}$ ). (D-F) (Original magnification 200x. Scale bar 50  $\mu\text{m}$ ).

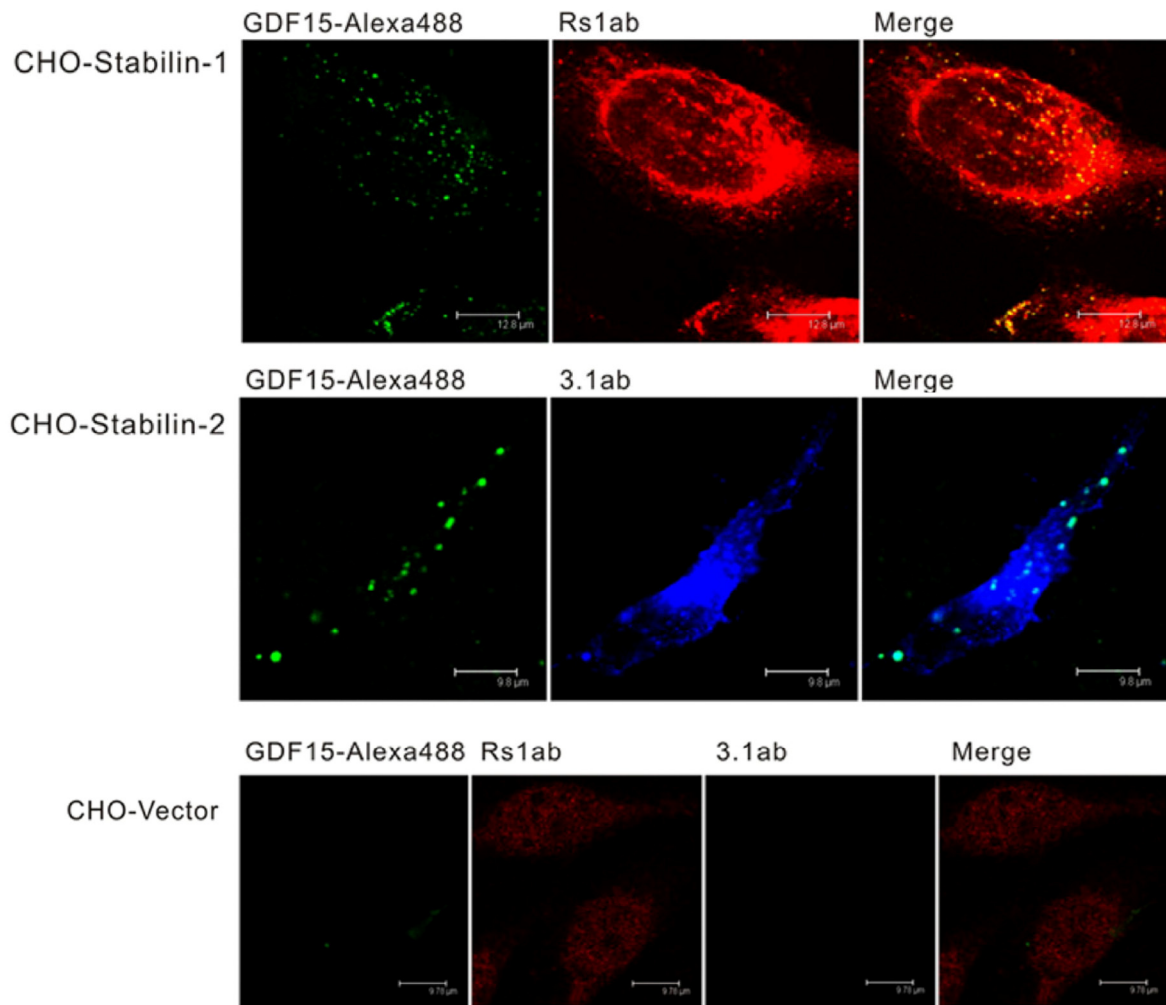
(G) Stabilin-1 positive cells were stained in green, whereas CD31 positive blood vessels appear in red. No overlapping signals indicate absence of stabilin-1 expression in CD31 positive blood vessels. (Original magnification 630x. Scale bar 35  $\mu\text{m}$ ). (H) In contrast, when stabilin-1 expressing cells (green) were tested against CD68 stained macrophages (red) a dominant overlap was observed within a subset of CD68 positive macrophages indicating a (locally) restricted stabilin-1 positive perivascular macrophage subpopulation. (Original magnification 630x. Scale bar 12  $\mu\text{m}$ ).



**Supplementary Figure 4**

**Serum parameter analysis associated with fibrotic disease.**

(A) Plasma of 3, 6, and 12 month old WT, stab-1, stab-2, and stab-dko mice of C57BL/6 genetic background were measured for fibronectin by ELISA. Moderately elevated levels of fibronectin were detected in stab-dko (at all ages tested) and in stab-1 and stab-2 mice at 12 months. Data represent mean  $\pm$  SEM for at least 5 mice per genotype. \* $P < 0.05$ ; \*\* $P < 0.01$ . (B-E) Plasma of 3 and 12 month old WT, stab-1, stab-2, and stab-dko mice of C57BL/6 genetic background were measured for IGF-1 (B) and PDFG-A (C) IGFBP2 (D), and SPARC (E) by ELISA. No elevated levels of profibrotic cytokines were detected in the tested stabilin animals: IGF-1 (B) showed a reduction in stab-2 and stab-dko. Data represent mean  $\pm$  SEM for at least 5 mice per genotype. \*\* $P < 0.01$ .



### Supplementary Figure 5

#### **Both stabilin-1 and stabilin-2 endocytose recombinant gdf-15.**

Visualisation of endocytic localisation of Alexa488-GDF-15 using immunofluorescence and confocal microscopy. Alexa488-GDF-15 is shown in green, stabilin-1 is shown in red, and stabilin-2 is shown in blue. Alexa488-GDF-15 co-localised both with stabilin-1 and stabilin-2 in endosomes. No signal in endosomes was observed in control CHO-vector cells. (n=3). Original Magnification 630x. Scale bars from top to bottom 12.8 μm; 9.8 μm; 9.78 μm.

**Supplementary Table 1:**

Survival analysis of all C57BL/6 and Balb/c stabilin knockout mouse variants. Animal groups described in detail in legend to Figure 1

<b>C57BL/6</b>	<b>Mean Survival [d]</b>	<b>Std. Error [d]</b>	<b>95% Conf. Lower</b>	<b>95% Conf. Upper</b>
WT	803.500	21.082	762.181	844.819
Stab-1	614.982	26.181	563.667	666.296
Stab-2	624.727	28.372	569.118	680.336
Stab-dko	415.159	30.459	355.460	474.858
<b>Comparisons</b>	<b>Statistic</b>	<b>Unadjusted P Value</b>	<b>Critical Level</b>	<b>Significant? *</b>
WT vs. Stab-dko	32.474	0.0000000121	0.00851	Yes
Stab-1 vs. Stab-dko	20.492	0.00000599	0.0102	Yes
Stab-2 vs. Stab-dko	19.792	0.00000864	0.0127	Yes
WT vs. Stab-2	2.481	0.115	0.0170	No
WT vs. Stab-1	2.325	0.127	0.0253	No
Stab-1 vs. Stab-2	0.0146	0.904	0.0500	No

<b>Balb/c</b>	<b>Mean Survival [d]</b>	<b>Std. Error [d]</b>	<b>95% Conf. Lower</b>	<b>95% Conf. Upper</b>
WT	738.746	11.975	715.276	762.216
Stab-1	584.037	16.455	551.786	616.289
Stab-2	617.874	26.686	565.570	670.179
Stab-dko	269.359	16.960	236.119	302.600
<b>Comparisons</b>	<b>Statistic</b>	<b>Unadjusted P Value</b>	<b>Critical Level</b>	<b>Significant? *</b>
WT vs. Stab-dko	59.072	1.520E-014	0.00851	Yes
Stab-1 vs. Stab-dko	46.790	7.901E-012	0.0102	Yes
Stab-2 vs. Stab-dko	36.905	0.00000000124	0.0127	Yes
WT vs. Stab-2	3.631	0.0567	0.0170	No
WT vs. Stab-1	1.178	0.278	0.0253	No
Stab-1 vs. Stab-2	0.374	0.541	0.0500	No

\* All Pairwise Multiple Comparison Procedures (Holm-Sidak method): Overall significance level = 0.05



**Supplementary Table 2:**

**Metabolic and physiological analysis of Stabilin-deficient animals.**

WT, stab-1, stab-2, stab-dko animals of the same genetic background (Balb/c or C57BL/6) were arranged in groups of 5-10 and followed for an observation period of up to 2 years. The table lists the values measured for a group of WT, stab-1, stab-2, stab-dko of Balb/c background measured at 6 months of age. No differences are found in mice of C57BL/6. Statistical analysis revealed no relevant differences except values measured only for urine albumine concentrations. Data represent mean ± SEM. (n=10). \*\**P* < 0.01.

	Balb/c WT		Balb/c stab-1		Balb/c stab-2		Balb/c stab-dko	
	MEAN	+/-	MEAN	+/-	MEAN	+/-	MEAN	+/-
physiologic cage								
food/24h [g]	3.69	1.05	3.93	1.38	4.01	0.62	3.53	0.63
water/24h [g]	3.54	1.27	3.10	1.58	3.16	0.51	4.22	0.87
urine/24h [g]	1.12	0.40	0.90	0.24	0.82	0.14	1.50	0.84
feces/24h [g]	1.54	0.60	1.96	0.54	1.58	0.50	1.30	0.26
animal weigth [g]	25.48	2.09	22.64	0.40	24.50	2.04	23.60	0.94
Urine parameter								
protein [mg/l]	2.43	0.83	3.80	1.19	2.48	0.59	6.81	7.03
creatinine [mg/dl]	43.89	18.14	48.07	13.18	46.83	8.95	43.70	19.31
urea [mg/dl]	11356.30	5514.08	12131.30	5750.65	16992.90	5165.09	16908.80	6973.46
glucose [mg/dl]	86.30	42.77	79.40	26.42	92.60	17.95	69.30	35.78
Albumin [mg/l]	26.50	7.49	30.50	9.20	35.10	26.47	1031.00 **	1456.13
Na [mmol/l]	220.40	86.33	214.00	94.19	303.60	59.70	207.50	108.15
K [mmol/l]	494.92	178.94	590.62	177.14	647.04	90.90	502.95	188.52
Ca [mmol/l]	3.20	1.05	3.45	0.86	3.79	0.46	3.68	1.28
PO4 [mmol/l]	129.80	71.57	125.64	39.91	85.66	25.87	87.50	44.93
plasma parameter								
creatinin [mg/dl]	0.08	0.02	0.09	0.02	0.08	0.02	0.10	0.02
urea [mg/dl]	73.21	16.51	71.35	10.43	53.32	7.47	58.03	8.48
protein [g/l]	51.10	1.90	48.67	1.43	49.56	1.74	46.88	1.58
Albumin [g/l]	33.25	7.073	27.25	3.092	39.88	5.52	24.00	3.91
cholin esterase [u/l]	8005.30	706.61	6859.40	543.59	6772.20	428.96	6873.10	582.66
Glucose [mg/dl]	158.80	15.65	148.10	12.84	167.40	17.58	159.70	13.74
Cholesterol [mg/dl]	81.10	8.75	98.80	10.92	80.60	8.63	92.20	14.63
Triglyc.[mg/dl]	90.00	26.41	68.10	14.75	84.60	24.34	79.80	29.87
AST [u/l]	76.07	11.45	97.27	25.39	75.01	14.44	73.37	11.00
ALT [u/l]	41.38	16.08	79.50	47.26	42.22	32.48	64.22	34.98
GLDH [u/l]	13.69	6.44	14.49	3.72	9.36	1.41	16.50	18.27
ggt [u/l]	3.00	0.00	3.00	0.00	3.00	0.00	3.00	0.00