

Supplementary Materials

Table S1. List of common reference housekeeping genes based on a targeted literature search conducted using the PubMed database (2013–2023) involving qPCR data normalization studies across diverse spectrums of tissues, disease models, and animal species. Values indicate the number of peer-reviewed articles that mentioned or used a particular HKG in the context of qPCR data normalization.

	“Housekeeping gene”	“Reference”	“Internal control”	“qPCR reference”
<i>Gapdh</i>	513	1053	398	499
<i>Actb</i>	202	433	81	256
<i>Tbp</i>	140	311	54	139
<i>B2m</i>	132	277	59	161
<i>Hprt1</i>	101	206	33	123
<i>Pgk1</i>	50	77	14	41
<i>Rplp0</i>	40	70	12	43
<i>Tfrc</i>	35	49	8	23
<i>Rplp2</i>	2	7	0	4

Table S2. List and characteristics of antibodies used in this study.

Antibody	Brand	Catalog number	Dilution used
ACTB	Abcam	Ab49900	1:30,000
B2M	Novus Biologicals	NBP2-92971	1:500
GAPDH	Cell Signaling	5174	1:5,000
HPRT1	Novus Biologicals	NBP1-92006	1:1,000
PGK1	Santa Cruz	SC-130335	1:3,000
RPLP0	Abcam	AB192866	1:800
RPLP2	Novus Biologicals	NBP2-33688	1:800
TBP	Cell Signaling	8515S	1:800
TFRC	Novus Biologicals	NB100-92243SS	1:800

Table S3. Calculated mean of Ct values, standard deviations and coefficients of gene expression variation (CV) for the 9 housekeeping genes under steady state and post- injury in lung, liver, kidney and skeletal muscle (n ≥ 4 animals/group).

Lung	Naïve	6 h Post-Injury	24 h Post-Injury	168 h Post-Injury	All Injury time-points	Naïve+ All time-points
<i>Actb</i>	23.77 ± 2.22 CV: 0.093	29.87 ± 3.97 CV: 0.133	24.54 ± 1.13 CV: 0.046	24.64 ± 0.81 CV: 0.033	26.25 ± 3.40 CV: 0.130	25.09 ± 3.16 CV: 0.126
<i>B2m</i>	20.50 ± 1.87 CV: 0.091	25.03 ± 2.66 CV: 0.106	20.77 ± 1.59 CV: 0.077	20.86 ± 1.04 CV: 0.050	21.94 ± 2.58 CV: 0.118	21.25 ± 2.38 CV: 0.112
<i>Gapdh</i>	24.46 ± 1.42 CV: 0.058	26.67 ± 1.38 CV: 0.052	23.65 ± 1.63 CV: 0.069	24.76 ± 0.76 CV: 0.031	24.90 ± 1.73 CV: 0.069	24.69 ± 1.60 CV: 0.065
<i>Hprt1</i>	25.08 ± 1.42 CV: 0.057	27.41 ± 1.93 CV: 0.070	24.18 ± 0.86 CV: 0.036	24.64 ± 0.78 CV: 0.032	25.27 ± 1.85 CV: 0.073	25.17 ± 1.65 CV: 0.066
<i>Pgk1</i>	21.64 ± 0.55 CV: 0.025	22.22 ± 1.80 CV: 0.081	20.89 ± 0.46 CV: 0.022	21.70 ± 0.33 CV: 0.015	21.61 ± 1.19 CV: 0.055	21.62 ± 0.96 CV: 0.044

<i>Rplp0</i>	19.38 ± 1.12 CV: 0.058	22.3 ± 2.82 CV: 0.126	18.87 ± 0.74 CV: 0.039	19.18 ± 0.49 CV: 0.026	20.06 ± 2.25 CV: 0.112	19.74 ± 1.85 CV: 0.094
<i>Rplp2</i>	22.61 ± 1.63 CV: 0.072	26.83 ± 3.10 CV: 0.116	22.33 ± 1.02 CV: 0.046	22.55 ± 0.64 CV: 0.028	23.82 ± 2.76 CV: 0.116	23.26 ± 2.38 CV: 0.102
<i>Tbp</i>	27.71 ± 2.17 CV: 0.078	31.58 ± 2.35 CV: 0.074	27.78 ± 0.92 CV: 0.033	28.58 ± 0.76 CV: 0.027	29.11 ± 2.08 CV: 0.071	28.44 ± 2.23 CV: 0.078
<i>Tfrc</i>	28.13 ± 1.55 CV: 0.055	30.06 ± 0.96 CV: 0.032	28.03 ± 1.01 CV: 0.036	29.08 ± 0.49 CV: 0.017	28.99 ± 1.14 CV: 0.039	28.59 ± 1.41 CV: 0.049
Liver	Naïve	6 h Post-Injury	24 h Post-Injury	168 h Post-Injury	All Injury time-points	Naïve+ All time-points
<i>Actb</i>	25.44 ± 1.31 CV: 0.051	29.92 ± 5.08 CV: 0.170	29.12 ± 3.95 CV: 0.136	30.75 ± 5.11 CV: 0.166	29.93 ± 4.79 CV: 0.160	28.05 ± 4.35 CV: 0.155
<i>B2m</i>	20.59 ± 1.25 CV: 0.061	23.71 ± 2.94 CV: 0.124	23.28 ± 1.94 CV: 0.083	24.84 ± 3.34 CV: 0.134	23.94 ± 2.88 CV: 0.120	22.54 ± 2.85 CV: 0.126
<i>Gapdh</i>	22.86 ± 1.41 CV: 0.062	22.56 ± 1.10 CV: 0.049	22.92 ± 0.73 CV: 0.032	25.33 ± 1.34 CV: 0.053	23.61 ± 1.64 CV: 0.069	23.29 ± 1.59 CV: 0.068
<i>Hprt1</i>	25.55 ± 1.07 CV: 0.042	25.14 ± 1.02 CV: 0.041	25.44 ± 0.32 CV: 0.013	28.93 ± 2.84 CV: 0.098	26.50 ± 2.46 CV: 0.093	26.10 ± 2.05 CV: 0.079
<i>Pgk1</i>	21.91 ± 0.51 CV: 0.023	21.28 ± 0.75 CV: 0.035	20.83 ± 0.31 CV: 0.015	24.66 ± 3.11 CV: 0.126	22.26 ± 2.52 CV: 0.113	22.11 ± 1.96 CV: 0.089
<i>Rplp0</i>	20.90 ± 0.58 CV: 0.028	18.58 ± 0.78 CV: 0.042	18.32 ± 0.38 CV: 0.021	23.03 ± 2.72 CV: 0.118	19.98 ± 2.71 CV: 0.136	20.37 ± 2.15 CV: 0.106
<i>Rplp2</i>	23.50 ± 1.00 CV: 0.043	21.43 ± 0.86 CV: 0.040	20.88 ± 0.94 CV: 0.045	25.75 ± 1.81 CV: 0.070	22.68 ± 2.52 CV: 0.111	23.03 ± 2.07 CV: 0.090
<i>Tbp</i>	29.25 ± 1.11 CV: 0.038	27.21 ± 0.67 CV: 0.025	27.51 ± 0.67 CV: 0.024	34.61 ± 4.50 CV: 0.130	29.78 ± 4.33 CV: 0.145	29.56 ± 3.38 CV: 0.114
<i>Tfrc</i>	27.86 ± 1.35 CV: 0.048	26.98 ± 0.85 CV: 0.032	25.84 ± 0.78 CV: 0.030	32.54 ± 4.18 CV: 0.128	28.46 ± 3.85 CV: 0.135	28.21 ± 3.07 CV: 0.109
Kidney	Naïve	6 h Post-Injury	24 h Post-Injury	168 h Post-Injury	All Injury time-points	Naïve+ All time-points
<i>Actb</i>	25.96 ± 1.47 CV: 0.057	25.33 ± 1.02 CV: 0.040	29.51 ± 7.47 CV: 0.253	25.95 ± 0.93 CV: 0.036	26.93 ± 4.75 CV: 0.176	26.52 ± 3.78 CV: 0.143
<i>B2m</i>	24.07 ± 1.35 CV: 0.056	23.49 ± 1.33 CV: 0.057	23.25 ± 3.50 CV: 0.151	23.67 ± 0.48 CV: 0.020	23.47 ± 2.19 CV: 0.093	23.72 ± 1.90 CV: 0.080
<i>Gapdh</i>	23.38 ± 1.15 CV: 0.049	22.71 ± 0.73 CV: 0.032	21.72 ± 0.86 CV: 0.040	24.10 ± 0.69 CV: 0.029	22.84 ± 1.24 CV: 0.054	23.06 ± 1.24 CV: 0.054
<i>Hprt1</i>	27.32 ± 0.94 CV: 0.034	25.70 ± 0.79 CV: 0.031	24.74 ± 0.72 CV: 0.029	27.82 ± 0.72 CV: 0.026	26.09 ± 1.48 CV: 0.057	26.60 ± 1.42 CV: 0.053
<i>Pgk1</i>	22.29 ± 0.56 CV: 0.025	20.52 ± 0.66 CV: 0.032	19.95 ± 0.31 CV: 0.016	22.60 ± 0.51 CV: 0.023	21.03 ± 1.25 CV: 0.059	21.56 ± 1.20 CV: 0.056
<i>Rplp0</i>	21.34 ± 0.58 CV: 0.027	19.43 ± 0.66 CV: 0.034	18.63 ± 0.36 CV: 0.019	20.98 ± 0.46 CV: 0.022	19.68 ± 1.10 CV: 0.056	20.38 ± 1.23 CV: 0.060
<i>Rplp2</i>	23.76 ± 0.74 CV: 0.031	22.22 ± 0.69 CV: 0.031	21.52 ± 0.59 CV: 0.027	23.95 ± 0.69 CV: 0.029	22.57 ± 1.22 CV: 0.054	23.07 ± 1.20 CV: 0.052
<i>Tbp</i>	30.26 ± 1.20 CV: 0.040	28.25 ± 0.65 CV: 0.023	27.82 ± 0.34 CV: 0.012	30.69 ± 0.45 CV: 0.015	28.92 ± 1.36 CV: 0.047	29.45 ± 1.45 CV: 0.049
<i>Tfrc</i>	28.63 ± 1.19 CV: 0.042	26.92 ± 0.83 CV: 0.031	26.25 ± 0.27 CV: 0.010	29.67 ± 0.70 CV: 0.024	27.61 ± 1.61 CV: 0.058	28.04 ± 1.54 CV: 0.055
Muscle	Naïve	6 h Post-Injury	24 h Post-Injury	168 h Post-Injury	All Injury time-points	Naïve+ All time-points
<i>Actb</i>	28.85 ± 2.93 CV: 0.102	26.42 ± 1.83 CV: 0.069	26.10 ± 1.31 CV: 0.050	23.86 ± 1.06 CV: 0.044	25.46 ± 1.83 CV: 0.072	26.67 ± 2.80 CV: 0.105
<i>B2m</i>	24.77 ± 3.10 CV: 0.125	22.35 ± 1.73 CV: 0.077	22.08 ± 1.27 CV: 0.058	19.72 ± 0.99 CV: 0.050	21.38 ± 1.81 CV: 0.085	22.59 ± 2.86 CV: 0.127
<i>Gapdh</i>	21.38 ± 3.73 CV: 0.174	19.47 ± 2.65 CV: 0.136	19.20 ± 1.29 CV: 0.067	18.94 ± 1.08 CV: 0.057	19.20 ± 1.83 CV: 0.095	19.98 ± 2.86 CV: 0.143
<i>Hprt1</i>	26.95 ± 3.40	25.13 ± 2.50	23.93 ± 1.52	23.39 ± 0.73	24.15 ± 1.89	25.15 ± 2.87

	CV: 0.126	CV: 0.099	CV: 0.064	CV: 0.031	CV: 0.078	CV: 0.114
<i>Pgk1</i>	19.18 ± 1.17 CV: 0.061	19.39 ± 1.81 CV: 0.093	18.97 ± 0.50 CV: 0.026	20.11 ± 0.80 CV: 0.040	19.49 ± 1.27 CV: 0.065	19.38 ± 1.24 CV: 0.064
<i>Rplp0</i>	21.15 ± 2.34 CV: 0.111	20.48 ± 1.85 CV: 0.090	18.82 ± 0.80 CV: 0.043	18.54 ± 0.50 CV: 0.027	19.28 ± 1.47 CV: 0.076	19.95 ± 2.04 CV: 0.102
<i>Rplp2</i>	24.64 ± 2.89 CV: 0.117	22.78 ± 1.98 CV: 0.087	22.09 ± 1.33 CV: 0.060	20.90 ± 0.55 CV: 0.026	21.92 ± 1.61 CV: 0.073	22.89 ± 2.52 CV: 0.110
<i>Tbp</i>	30.92 ± 3.27 CV: 0.106	29.49 ± 2.53 CV: 0.086	28.62 ± 1.37 CV: 0.048	27.36 ± 0.56 CV: 0.020	28.49 ± 1.91 CV: 0.067	29.36 ± 2.74 CV: 0.093
<i>Tfrc</i>	27.01 ± 3.42 CV: 0.127	27.29 ± 2.02 CV: 0.074	28.71 ± 1.02 CV: 0.036	26.35 ± 0.88 CV: 0.033	27.45 ± 1.70 CV: 0.062	27.29 ± 2.47 CV: 0.091

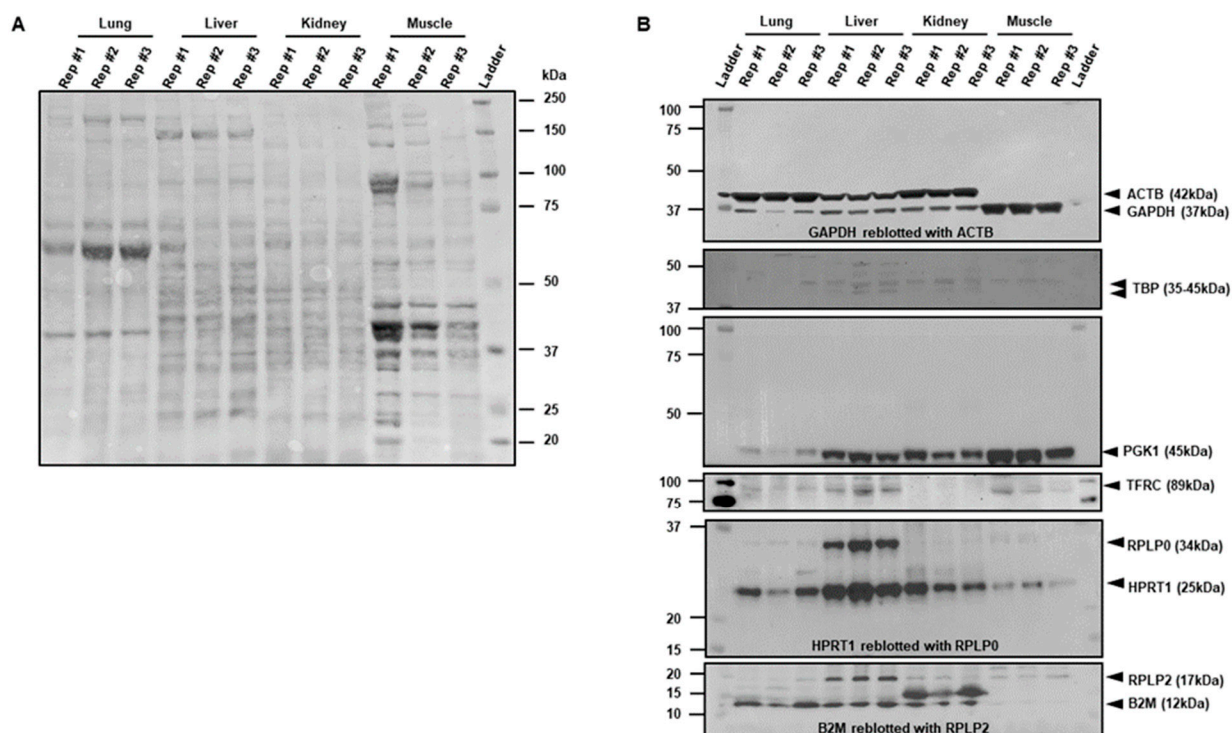


Figure S1. Representative Ponceau S and Western blot images. **(A)** Representative Ponceau S total protein staining following protein transfer from SDS-Page gels to nitrocellulose membranes. **(B)** Immunoblots against 9 HKGs for naïve lung, liver, kidney and muscle tissue (15 µg of total protein loaded lane; n = 3 biological replicates indicated as #1, #2 and #3).

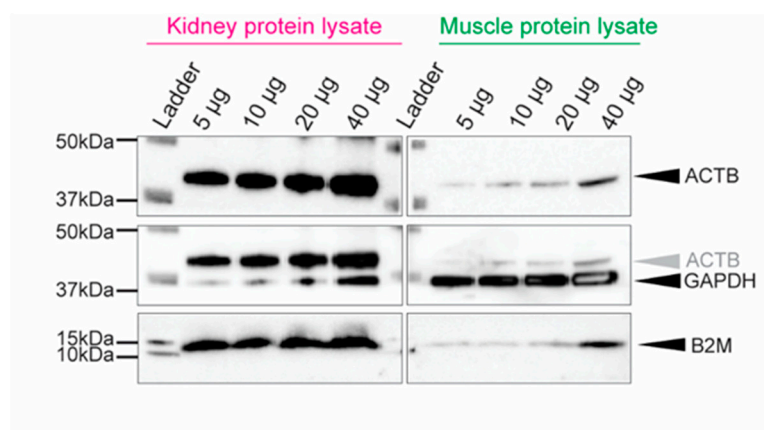


Figure S2. Immunoblots against ACTB, GAPDH and B2M using different concentrations of total protein from kidney and skeletal muscle tissue lysates. Note: ACTB membrane was re-probed for GAPDH.

LUNG		LIVER		KIDNEY		MUSCLE		
Genes	Geomean of ranking values NAIVE+INJURED	Genes	Geomean of ranking values NAIVE+INJURED	Genes	Geomean of ranking values NAIVE+INJURED	Genes	Geomean of ranking values NAIVE+INJURED	
<i>Rplp2/Tfrc/Rplp0</i>	1.41	<i>Hprt1/Pgk1/Tbp</i>	1.41	<i>Pgk1/Rplp0/Rplp2</i>	1.41	<i>Rplp0/Rplp2/Tbp</i>	1.41	Most stable
<i>Rplp2/Rplp0</i>	1.68	<i>Pgk1/Tbp</i>	2.11	<i>Pgk1/Rplp2</i>	1.68	<i>Rplp0/Tbp</i>	1.86	
<i>Rplp0/Tfrc</i>	3	<i>Hprt1/Pgk1</i>	2.91	<i>Rplp0/Rplp2</i>	3	<i>Rplp0/Rplp2</i>	2.71	
<i>Rplp0</i>	3.34	<i>Pgk1</i>	3.83	<i>Rplp2</i>	4.3	<i>Rplp0</i>	4.3	Least stable
<i>Rplp2/Tfrc</i>	4.43	<i>Hprt1/Tbp</i>	4.12	<i>Pgk1/Rplp0</i>	4.43	<i>Rplp2</i>	4.47	
<i>Rplp2</i>	6.24	<i>Hprt1</i>	4.40	<i>Rplp0</i>	5.44	<i>Rplp2/Tbp</i>	4.95	
<i>Tfrc</i>	6.44	<i>Tbp</i>	7	<i>Pgk1</i>	5.73	<i>Tbp</i>	6.24	

Figure S3. Gene stability ranking obtained with RefFinder using the three most stable HKG by comparing either single HKG or HKG combinations of two or three genes. The color-scale represents the most (yellow) to least (blue) stable HKG.