Supporting Information

Adsorptive microtiter plates as solid supports in affinity purification workflows

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Supplementary Method S1. Liquid chromatography-mass spectrometry (LC-MS) method for quantification of the soluble receptor for advanced glycation end-products (sRAGE) in human serum based on microtiter plate-based affinity purification and in-well proteolytic digestion.

Serum samples
An aliquot of pooled human serum from healthy subjects (Seralab) was used for preparation of the quality control (QC) samples. The pooled serum was either diluted eight times with 1% bovine serum albumin (BSA; Sigma-Aldrich, #A7638) in 10 mM phosphate buffered saline (PBS; Sigma-Aldrich, #D1408) to obtain the QC-low sample, was used directly as QC-medium sample, or was fortified with 3 ng/mL recombinant human sRAGE (Novoprotein, #C423; UniProtKB ID ‘Q15109’; Ala23-Ala344 with C-terminal hexa-histidine tag) to obtain the QC-high sample. Six different sources of human serum from healthy subjects (all from Seralab) were furthermore used for the recovery and spike recovery assessments.

Calibration and internal standard solutions
Calibration and internal standard solutions were prepared as described previously in the original manuscript which outlines the quantitative sRAGE method featuring an elution step after microtiter plate-based affinity purification of serum sRAGE (see Klont, F. & Pouwels, S.D., et al. *Talanta* **182**, 414-421 (2018)).

Sample preparation protocol
- Add 100 µL 5 µg/mL anti-RAGE monoclonal antibody (R&D Systems, #MAB11451) in 10 mM phosphate buffered saline (PBS; Sigma-Aldrich, #D1408) to the wells of a Nunc-Immuno™ MicroWell™ 96 wells plate with MaxiSorp™ coating (Sigma-Aldrich, #M9410), and let the antibodies adsorb to the plate upon overnight incubation at room temperature;
- Wash the wells 3 times with 300 µL wash buffer containing 0.05% Tween-20 (Sigma-Aldrich, #P5927) in 10 mM PBS to remove unbound antibody;
- Add 300 µL blocking buffer containing 1% bovine serum albumin (BSA; Sigma-Aldrich, #A7638) in 10 mM PBS to each well, and incubate for 1 hour at room temperature on a plate shaker shaking at 600 RPM to block uncoated surfaces;
- Wash the wells 3 times with 300 µL wash buffer to remove unbound BSA;
- Add 100 µL of a 1:1 mixture of serum/calibrant and blocking buffer to each well, and incubate for 2 hours at room temperature on a plate shaker shaking at 600 RPM to let sRAGE proteins bind to the immobilized anti-sRAGE antibodies;
- Wash the wells 3 times with 300 µL wash buffer to remove the unbound fraction;
- Add 100 µL 10 mM tris(2-carboxyethyl)phosphine (TCEP; Sigma-Aldrich, #C4706) and 50 pM stable isotope labelled (SIL) sRAGE peptides (i.e. IGEPLVL[13C15N-lysine] and VLSPQGGGPDSVA[13C15N-arginine], both synthesized by Pepscan Presto) in 50 mM ammonium bicarbonate (ABC; Sigma-Aldrich, #A6141) to each well, and incubate for 30 minutes at room temperature on a plate shaker shaking at 600 RPM to reduce the disulfide bonds;
- Add 5 µl 420 mM iodoacetamide (IAM; Sigma-Aldrich, #I1149) in 50 mM ABC, and incubate in the dark (in a drawer) for 30 minutes at room temperature to alkylate the thiols;
- Add 5 µl 210 mM dithiothreitol (DTT; Sigma-Aldrich, #D9779) in 50 mM ABC, and incubate for 5 minutes at room temperature to quench unreacted IAM;
- Add 2 µL 50 ng/µL sequencing grade modified trypsin (Promega, #V511A) in 50 mM ABC, and incubate overnight at 37 °C in an oven to digest the proteins;
- Add 5 µL 25% formic acid (FA; Sigma-Aldrich, #F0507) to stop digestion, and inject 20 µL of the resulting peptide mixture into the LC-MS system.
**LC-MS analysis**
Analyses were performed with a Waters Ionkey/MS system using an ACQUITY M-Class UPLC and a XEVO TQ-S mass spectrometer operating in the single reaction monitoring (SRM) mode, as described previously in the original manuscript which outlines the quantitative sRAGE method featuring an elution step after microtiter plate-based affinity purification of serum sRAGE (see Klont, F. & Pouwels, S.D., et al. *Talanta* **182**, 414-421 (2018)).

**Method validation**
In accordance with current guidelines on bioanalytical method validation (*i.e.* Food and Drug Administration (FDA). *Bioanalytical Method Validation: Guidance for Industry* (Center for Drug Evaluation and Research, Silver Spring MD, 2018)), the novel method was evaluated by means of a partial validation procedure, since it represents a modified version of the original method by featuring “Changes in sample preparation procedures”. Criteria that were addressed, include: selectivity (*e.g.* spike recovery), accuracy & precision, recovery, and calibration curve. A detailed overview of how these criteria were assessed, can be found in the original manuscript which describes the quantitative sRAGE method featuring an elution step after microtiter plate-based affinity purification of serum sRAGE (see Klont, F. & Pouwels, S.D., et al. *Talanta* **182**, 414-421 (2018)).
Supplementary Method S2. Identification of Transcription factor p65 (NF-κB) in murine RAW 264.7 leukemic macrophage cell lysates after microtiter plate-based affinity purification and liquid chromatography-mass spectrometry (LC-MS) analysis using an Orbitrap Q Exactive Plus mass spectrometer operating in the data-dependent acquisition (DDA) mode.

Cell culturing and lysis
Murine RAW 264.7 leukemic macrophages (American Type Culture Collection, #TIB-71) were cultured in plastic culture flasks at 37 °C under 5% carbon dioxide/95% air in Dulbecco’s Modification of Eagle’s Medium (DMEM) supplemented with 10% (v/v) heat inactivated fetal bovine serum (FBS), 2 mM GlutaMAX™, 100 U/mL penicillin, and 100 μg/mL streptomycin, as described previously (see Leus, N.G.J., et al. Biochemical Pharmacology 108, 58-74 (2016)). RAW 264.7 macrophages (between passage 5 and 9) were lysed using an ice-cold cell lysis buffer containing 25 mM 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES), 5 mM magnesium chloride, 5 mM ethylenediaminetetraacetic acid (EDTA), 0.5% (v/v) Triton X-100, 1 mM dithiothreitol (DTT), 1 mM sodium butyrate, and 1× Protease Inhibitor, followed by 4 freeze-thaw cycles and 10 minutes of centrifugation (13,000×g; 4 °C) to remove cell debris, as described previously (see Leus, N.G.J., et al. Biochemical Pharmacology 108, 58-74 (2016)).

Microtiter plate-based affinity purification and proteolytic digestion of Transcription factor p65 (NF-κB)
- Add 100 µL 5 µg/mL anti-NF-κB monoclonal antibody (Cell Signaling, #8242S) in 10 mM phosphate buffered saline (PBS; Sigma-Aldrich, #D1408) to the wells of a Nunc-Immuno™ MicroWell™ 96 wells plate with MaxiSorp™ coating (Sigma-Aldrich, #M9410), and let the antibodies adsorb to the plate upon overnight incubation at room temperature;
- Wash the wells 3 times with 300 µL 10 mM PBS to remove unbound antibody;
- Add 300 µL blocking buffer containing 1% bovine serum albumin (BSA; Sigma-Aldrich, #A7638) in 10 mM PBS to each well, and incubate for 1 hour at room temperature on a plate shaker shaking at 600 RPM to block uncoated surfaces;
- Wash the wells 3 times with 300 µL 10 mM PBS to remove unbound BSA;
- Add 100 µL of cell lysate to each well, and incubate for 2 hours at room temperature on a plate shaker shaking at 600 RPM to let NF-κB proteins bind to the immobilized anti-NF-κB antibodies;
- Wash the wells 3 times with 300 µL 10 mM PBS to remove the unbound fraction;
- Add 100 µL 0.1% trifluoroacetic acid (TFA; Sigma-Aldrich, #T6508) to each well, and incubate for 10 minutes at room temperature on a plate shaker shaking at 600 RPM to elute the captured proteins from the antibodies;
- Collect the eluates in low binding tubes (Eppendorf, #022431081) using low binding tips (VWR, #613-0891), and dry them in a vacuum centrifuge at 45 °C.
- Reconstitute the proteins in 50 µL 50 mM ammonium bicarbonate (ABC; Sigma-Aldrich, #A6141);
- Add 5 µl 110 mM dithiothreitol (DTT; Sigma-Aldrich, #D9779) in 50 mM ABC, and incubate for 30 minutes at 60 °C in a Thermomixer (Eppendorf) shaking at 600 RPM to reduce the disulfide bonds;
- Add 5 µl 240 mM iodoacetamide (IAM; Sigma-Aldrich, #I1149) in 50 mM ABC, and incubate in the dark (in a drawer) for 30 minutes at room temperature to alkylate the thiols;
- Add 6 µl 110 mM dithiothreitol (DTT; Sigma-Aldrich, #D9779) in 50 mM ABC, and incubate for 5 minutes at room temperature to quench unreacted IAM;
- Add 2 µL 50 ng/µL sequencing grade modified trypsin (Promega, #V511A) in 50 mM ABC, and incubate overnight at 37 °C in an oven to digest the proteins;
- Add 2 µL 50% formic acid (FA; Sigma-Aldrich, #F0507) to stop digestion, and inject 5 µL of the resulting peptide mixture into the LC-MS system.
**LC-MS analysis**

Analyses were performed using a Dionex UltiMate 3000 RSLC UHPLC system connected to a Thermo Fisher Scientific Orbitrap Q Exactive Plus mass spectrometer operating in the data-dependent acquisition (DDA) mode. A sample volume of 5 µL was injected onto an Acclaim PepMap100 C18 trap column (5 µm particles, 100 Å pore size, 300 µm × 5 mm; Dionex, #160454) using µL-pickup with 0.1% formic acid (FA) in H$_2$O at 20 µL/min. Peptides were separated on an Acclaim PepMap RSLC C18 analytical column (2 µm particles, 100 Å pore size, 75 µm × 500 mm; Dionex, #164540) at 40 °C using a 30-minute linear gradient from 1 to 30% eluent B (0.1% FA in acetonitrile) in eluent A (0.1% FA in H$_2$O) at a flow rate of 300 nL/min. For DDA, survey scans from 300 to 1,650 m/z were acquired at a resolution of 70,000 (at 200 m/z) with an AGC target value of $3 \times 10^6$ and a maximum ion injection time of 100 ms. From the survey scan, a maximum number of 10 of the most abundant precursor ions with a charge state of 2+ to 6+ were selected for fragmentation by higher energy collisional dissociation (HCD). Fragment ion spectra were acquired between 200 and 2,000 m/z at a resolution of 17,500 (at 200 m/z) with an AGC target value of $1 \times 10^5$, a maximum ion injection time of 200 ms, a normalized collision energy of 28%, an isolation window of 1.6 m/z, an underfill ratio of 1%, an intensity threshold of $5 \times 10^3$, and the dynamic exclusion parameter set at 15 s.

**Data processing**

DDA data were processed using PEAKS Studio software (version 8.5), and peak lists were searched against the UniProtKB mus musculus ‘UP000000589’ reference proteome (canonical; 33,960 entries; downloaded on August 6, 2018). Trypsin was selected as protease (≤3 missed cleavages), cysteine carbamidomethylation as fixed modification, and methionine oxidation as variable modifications. Up to 6 modifications per peptide, ≤10.0 ppm precursor mass deviation (using monoisotopic mass), ≤0.02 Da fragment ion mass deviation, and ≤0.1% false discovery rates (FDR) for peptide-spectrum matches (PSMs), peptides, and proteins were allowed, and at least one unique peptide was required for protein identification.

**Nota Bene**

Potential nonspecific NFκB enrichment had been assessed first upon performing the microtiter plate-based enrichment procedure on murine RAW 264.7 leukemic macrophages using non-specific antibodies (e.g. anti-human IGF1 polyclonal antibody (Cell Sciences, Cat. No. PA0362), anti-human sRAGE monoclonal antibody (R&D Systems, Cat. No. MAB11451)), which yielded no identification of NF-kB.
Supplementary Method S3. Identification of Peptidyl-prolyl cis-trans isomerase FKBP5 (FKBP5) in human A549 adenocarcinomic alveolar basal epithelial cell lysates after microtiter plate-based affinity purification and liquid chromatography-mass spectrometry (LC-MS) analysis using an Orbitrap Q Exactive Plus mass spectrometer operating in the data-dependent acquisition (DDA) mode.

Cell culturing and lysis

Human A549 adenocarcinomic alveolar basal epithelial cells (American Type Culture Collection, #CCL-185) were grown to confluence in plastic 12-wells plates at 37 °C under 5% carbon dioxide/95% air in Roswell Park Memorial Institute 1640 medium (RPMI-1640) supplemented with heat inactivated fetal bovine serum (FBS), 100 U/mL penicillin, and 100 µg/mL streptomycin. Subsequently, cells were serum-starved in RPMI-1640 medium supplemented with 100 U/mL penicillin and 100 µg/mL streptomycin for 24 hours, and cells were lysed using M-PER™ mammalian protein extraction reagent followed by 15 minutes of centrifugation (13,000×g; 4 °C) to remove cell debris.

Microtiter plate-based affinity purification and proteolytic digestion of Peptidyl-prolyl cis-trans isomerase FKBP5 (FKBP5)

- Add 100 µL 5 µg/mL anti-FKBP5 monoclonal antibody (R&D Systems, #MAB4094) in 10 mM phosphate buffered saline (PBS; Sigma-Aldrich, #D1408) to the wells of a Nunc-Immuno™ MicroWell™ 96 wells plate with MaxiSorp™ coating (Sigma-Aldrich, #M9410), and let the antibodies adsorb to the plate upon overnight incubation at room temperature;
- Wash the wells 3 times with 300 µL 10 mM PBS to remove unbound antibody;
- Add 300 µL blocking buffer containing 1% bovine serum albumin (BSA; Sigma-Aldrich, #A7638) in 10 mM PBS to each well, and incubate for 1 hour at room temperature on a plate shaker shaking at 600 RPM to block uncoated surfaces;
- Wash the wells 3 times with 300 µL 10 mM PBS to remove unbound BSA;
- Add 250 µL of cell lysate to each well, and incubate for 2 hours at room temperature on a plate shaker shaking at 600 RPM to let FKBP5 proteins bind to the immobilized anti-FKBP5 antibodies;
- Wash the wells 3 times with 300 µL 10 mM PBS to remove the unbound fraction;
- Add 100 µL 0.1% trifluoroacetic acid (TFA; Sigma-Aldrich, #T6508) to each well, and incubate for 10 minutes at room temperature on a plate shaker shaking at 600 RPM to elute the captured proteins from the antibodies;
- Collect the eluates in low binding tubes (Eppendorf, #022431081) using low binding tips (VWR, #613-0891), and dry them in a vacuum centrifuge at 45 °C.
- Reconstitute the proteins in 50 µL 50 mM ammonium bicarbonate (ABC; Sigma-Aldrich, #A6141);
- Add 5 µl 110 mM dithiothreitol (DTT; Sigma-Aldrich, #D9779) in 50 mM ABC, and incubate for 30 minutes at 60 °C in a Thermomixer (Eppendorf) shaking at 600 RPM to reduce the disulfide bonds;
- Add 5 µl 240 mM iodoacetamide (IAM; Sigma-Aldrich, #I1149) in 50 mM ABC, and incubate in the dark (in a drawer) for 30 minutes at room temperature to alkylate the thiols;
- Add 6 µl 110 mM dithiothreitol (DTT; Sigma-Aldrich, #D9779) in 50 mM ABC, and incubate for 5 minutes at room temperature to quench unreacted IAM;
- Add 2 µl 50 ng/µL sequencing grade modified trypsin (Promega, #V511A) in 50 mM ABC, and incubate overnight at 37 °C in an oven to digest the proteins;
- Add 2 µl 50% formic acid (FA; Sigma-Aldrich, #F0507) to stop digestion, and inject 6 µL of the resulting peptide mixture into the LC-MS system.
**LC-MS analysis**

Analyses were performed using a Dionex UltiMate 3000 RSLC UHPLC system connected to a Thermo Fisher Scientific Orbitrap Q Exactive Plus mass spectrometer operating in the data-dependent acquisition (DDA) mode. A sample volume of 6 µL was injected onto an Acclaim PepMap100 C18 trap column (5 µm particles, 100 Å pore size, 300 µm × 5 mm; Dionex, #160454) using µL-pickup with 0.1% formic acid (FA) in H₂O at 20 µL/min. Peptides were separated on an Acclaim PepMap RSLC C18 analytical column (2 µm particles, 100 Å pore size, 75 µm × 500 mm; Dionex, #164540) at 40 °C using a 70-minute linear gradient from 3 to 50% eluent B (0.1% FA in acetonitrile) in eluent A (0.1% FA in H₂O) at a flow rate of 300 nL/min. For DDA, survey scans from 300 to 1,650 m/z were acquired at a resolution of 70,000 (at 200 m/z) with an AGC target value of 3 × 10⁶ and a maximum ion injection time of 50 ms. From the survey scan, a maximum number of 10 of the most abundant precursor ions with a charge state of 2⁺ to 5⁺ were selected for fragmentation by higher energy collisional dissociation (HCD). Fragment ion spectra were acquired between 200 and 2,000 m/z at a resolution of 17,500 (at 200 m/z) with an AGC target value of 1 × 10⁵, a maximum ion injection time of 110 ms, a normalized collision energy of 28%, an isolation window of 1.6 m/z, a minimum ACG target value of 1 × 10⁴, an intensity threshold of 9.4 × 10⁴, and the dynamic exclusion parameter set at 15 s.

**Data processing**

DDA data were processed using PEAKS Studio software (version 8.5), and peak lists were searched against the UniProtKB homo sapiens ‘UP000005640’ reference proteome (canonical; 40,742 entries; downloaded on August 6, 2018). Trypsin was selected as protease (≤3 missed cleavages), cysteine carbamidomethylation as fixed modification, and methionine oxidation as variable modifications. Up to 6 modifications per peptide, ≤10.0 ppm precursor mass deviation (using monoisotopic mass), ≤0.02 Da fragment ion mass deviation, and ≤0.1% false discovery rates (FDR) for peptide-spectrum matches (PSMs), peptides, and proteins were allowed, and at least one unique peptide was required for protein identification.

**Nota Bene**

Human A549 alveolar basal epithelial cells which were CRISPR/Cas9-edited using the Px548 plasmid to knockout the gene FKBP5 (see Pouwels, S.D., et al. *Respirology* **26**, 233-240 (2021) were used for control experiments, which yielded no FKBP5 identification.
### Supplementary Table S1. Calibration curve results of the validation experiments for the quantitative method for serum sRAGE quantification.

| Run | 0.100 | 0.200 | 0.500 | 1.000 | 2.000 | 5.000 | 8.000 | 10.000 | a  | b             | R²     |
|-----|-------|-------|-------|-------|-------|-------|-------|--------|----|----------------|--------|
| 1   | 0.102 | 0.254 | 0.491 | 0.966 | 2.019 | 4.817 | 7.855 | 10.319 | 9.62 · 10⁻¹ | -1.31 · 10⁻³ | 0.9992 |
| 2   | 0.088 | 0.211 | 0.528 | 1.031 | 1.721 | 4.916 | 7.966 | 10.340 | 9.26 · 10⁻¹ | -1.03 · 10⁻² | 0.9977 |
| 3   | 0.097 | 3.652 | 0.519 | 0.930 | 1.914 | 5.106 | 7.610 | 10.423 | 1.01 · 10⁻⁰ | -8.71 · 10⁻³ | 0.9980 |

*Value was discarded as the back-calculated concentration was deviating > 15% from the nominal value.

### Supplementary Table S2. Accuracy and precision results for the lower limit of quantification (LLOQ) determination for the quantitative method for serum sRAGE quantification.

| Run | Replicate 1 | Replicate 2 | Replicate 3 | Replicate 4 | Replicate 5 | Average | CV  | Bias |
|-----|-------------|-------------|-------------|-------------|-------------|---------|-----|------|
| 1   | 0.102       | 0.101       | 0.119       | 0.099       | 0.111       | 0.106   | 8%  | 6%   |
| 2   | 0.088       | 0.118       | 0.126       | 0.134       | 0.111       | 0.115   | 15% | 15%  |
| 3   | 0.097       | 0.129       | 0.117       | 0.109       | 0.080       | 0.106   | 18% | 6%   |

Average: 0.109

### Supplementary Table S3. Accuracy and precision results for the low concentration validation sample (QC-low)* for the quantitative method for serum sRAGE quantification.

* The QC-low sample was prepared by diluting an aliquot of pooled human serum (from Seralab) eight times with 1% bovine serum albumin (BSA; Sigma-Aldrich, Cat. No. A7638) in 1× phosphate-buffered saline (PBS; Sigma-Aldrich, Cat. No. D1408).

| Run | Replicate 1 | Replicate 2 | Replicate 3 | Replicate 4 | Replicate 5 | Average | CV  | Bias** |
|-----|-------------|-------------|-------------|-------------|-------------|---------|-----|--------|
| 1   | 0.303       | 0.304       | 0.352       | 0.339       | 0.335       | 0.327   | 7%  | 1%     |
| 2   | 0.340       | 0.369       | 0.376       | 0.318       | 0.298       | 0.340   | 10% | 5%     |
| 3   | 0.295       | 0.329       | 0.305       | 0.308       | 0.276       | 0.303   | 6%  | -6%    |

Average: 0.323

** For calculating the bias, the average value of measured concentrations was used as nominal concentration.

### Supplementary Table S4. Accuracy and precision results for the medium concentration validation sample (QC-medium)* for the quantitative method for serum sRAGE quantification.

* An aliquot of pooled human serum (from Seralab) was used directly as QC-medium sample.

| Run | Replicate 1 | Replicate 2 | Replicate 3 | Replicate 4 | Replicate 5 | Average | CV  | Bias** |
|-----|-------------|-------------|-------------|-------------|-------------|---------|-----|--------|
| 1   | 2.843       | 3.132       | 2.847       | 3.125       | 2.854       | 2.960   | 5%  | 7%     |
| 2   | 2.672       | 2.806       | 2.945       | 2.735       | 2.890       | 2.810   | 4%  | 2%     |
| 3   | 2.295       | 2.442       | 2.675       | 2.678       | 2.581       | 2.534   | 6%  | -8%    |

Average: 2.768

** For calculating the bias, the average value of measured concentrations was used as nominal concentration.
Supplementary Table S5. Accuracy and precision results for the high concentration validation sample (QC-high)* for the quantitative method for serum sRAGE quantification.

| Run | Replicate 1 | Replicate 2 | Replicate 3 | Replicate 4 | Replicate 5 | Average | CV | Bias** |
|-----|-------------|-------------|-------------|-------------|-------------|---------|----|--------|
| 1   | 5.583       | 5.215       | 5.796       | 5.457       | 5.979       | 5.606   | 5% | -1%    |
| 2   | 6.159       | 5.974       | 5.558       | 6.032       | 5.699       | 5.884   | 4% | 3%     |
| 3   | 6.546       | 5.336       | 5.322       | 5.356       | 5.290       | 5.570   | 10%| -2%    |

Average: 5.687

* The QC-high sample was prepared by fortifying an aliquot of pooled human serum (from Seralab) with 3 ng/mL recombinant human sRAGE (Novoprotein; Cat. No. C423).

** For calculating the bias, the average value of measured concentrations was used as nominal concentration.

Supplementary Table S6. Recovery results for the quantitative method for serum sRAGE quantification.

| Run | Sample | Pre-IP spiked sample (+ 5 ng/mL) | Post-IP spiked sample (+ 5 ng/mL) | Recovery |
|-----|--------|----------------------------------|----------------------------------|----------|
| 3   | ♂      | 6.24                             | 6.98                             | 89%      |
| 3   | ♂      | 5.44                             | 6.43                             | 85%      |
| 3   | ♂      | 5.70                             | 5.59                             | 102%     |
| 3   | ♀      | 6.63                             | 6.81                             | 97%      |
| 3   | ♀      | 5.46                             | 5.42                             | 101%     |
| 3   | ♀      | 5.91                             | 6.20                             | 95%      |

Average: 95%

CV: 7%

Supplementary Table S7. Spike recovery results for the quantitative method for serum sRAGE quantification.

| Run | Sample | Non-spiked sample | Spiked sample (+ 5 ng/mL) | Bias |
|-----|--------|-------------------|----------------------------|------|
| 3   | ♂      | 1.30              | 6.05                       | -5%  |
| 3   | ♂      | 0.89              | 5.30                       | -12% |
| 3   | ♂      | 0.90              | 5.65                       | -5%  |
| 3   | ♀      | 1.95              | 6.43                       | -11% |
| 3   | ♀      | 0.42              | 5.58                       | 3%   |
| 3   | ♀      | 0.93              | 5.88                       | -1%  |

Average: -5%