Supplemental Information

A Connective Tissue Mast Cell-Specific Receptor Detects Bacterial Quorum Sensing Molecules and Mediates Antibacterial Immunity

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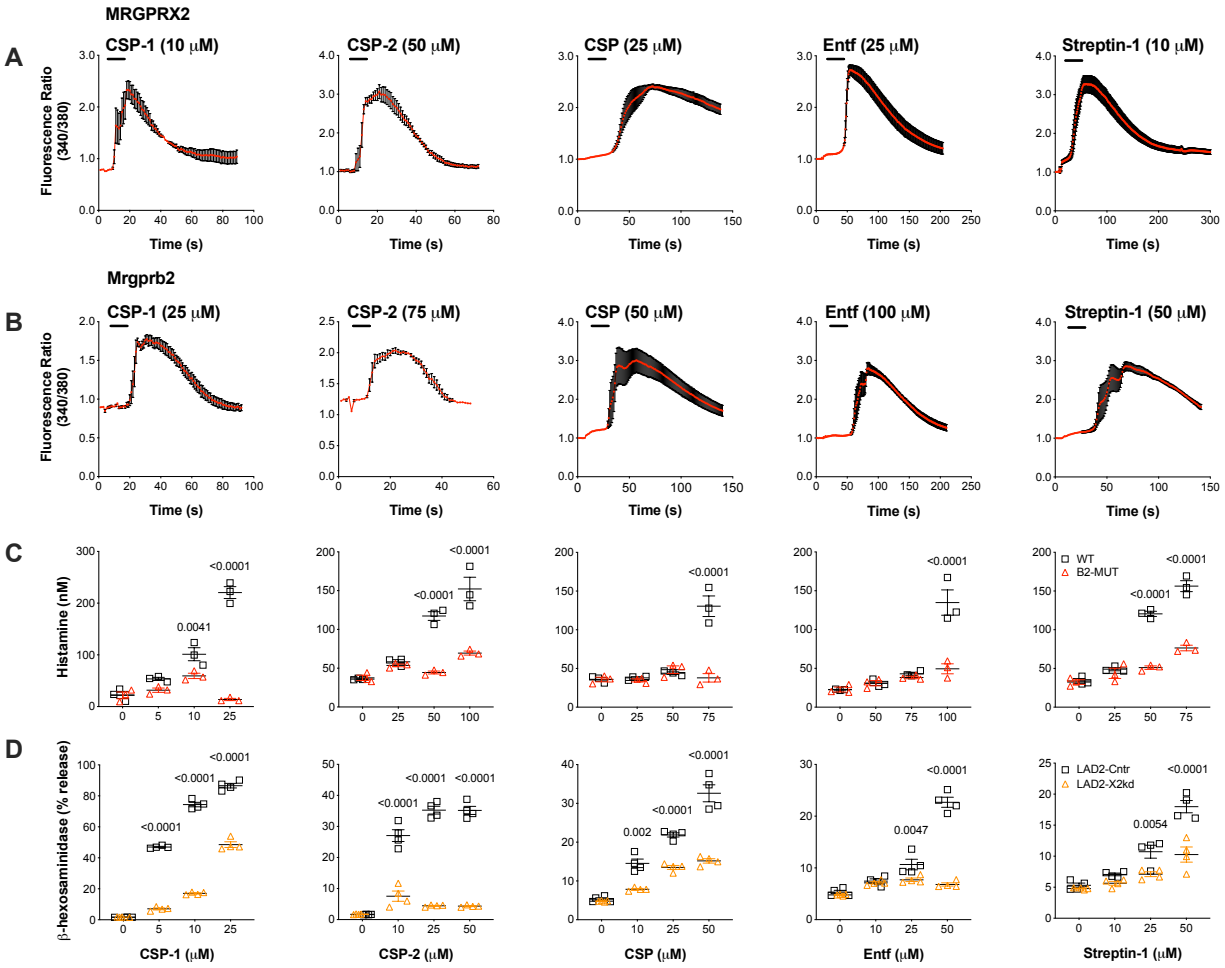

Figure S1. Bacterial peptides are agonists for MRGPRX2 and Mrgprb2, Related to Figure 1. (A, B) Example traces showing changes in intracellular calcium levels in Fura-2 loaded HEK293 cells transfected to stably express MRGPRX2 (**A**) or Mrgprb2 (**B**). The black line on the graphs indicates the application of bacterial product. The experiments were repeated independently at least five times with similar results. **(C, D)** Mrgprb2 and MRGPRX2 mediate mast cell responsiveness to peptide QSMs. (**C**) Histamine release from WT and Mrgprb2^{MUT} (B2-MUT) peritoneal mast cells following stimulation with peptide QSMs (n=3). (**D**) The release of β-hexosaminidase from LAD2 human mast cells transfected with control siRNA (LAD2-Cntr)

and MRGPRX2 siRNA (LAD2-X2kd) in response to QSMs (n=4). Mean ± SEM. Two-way ANOVA with Sidak's multiple comparison test.

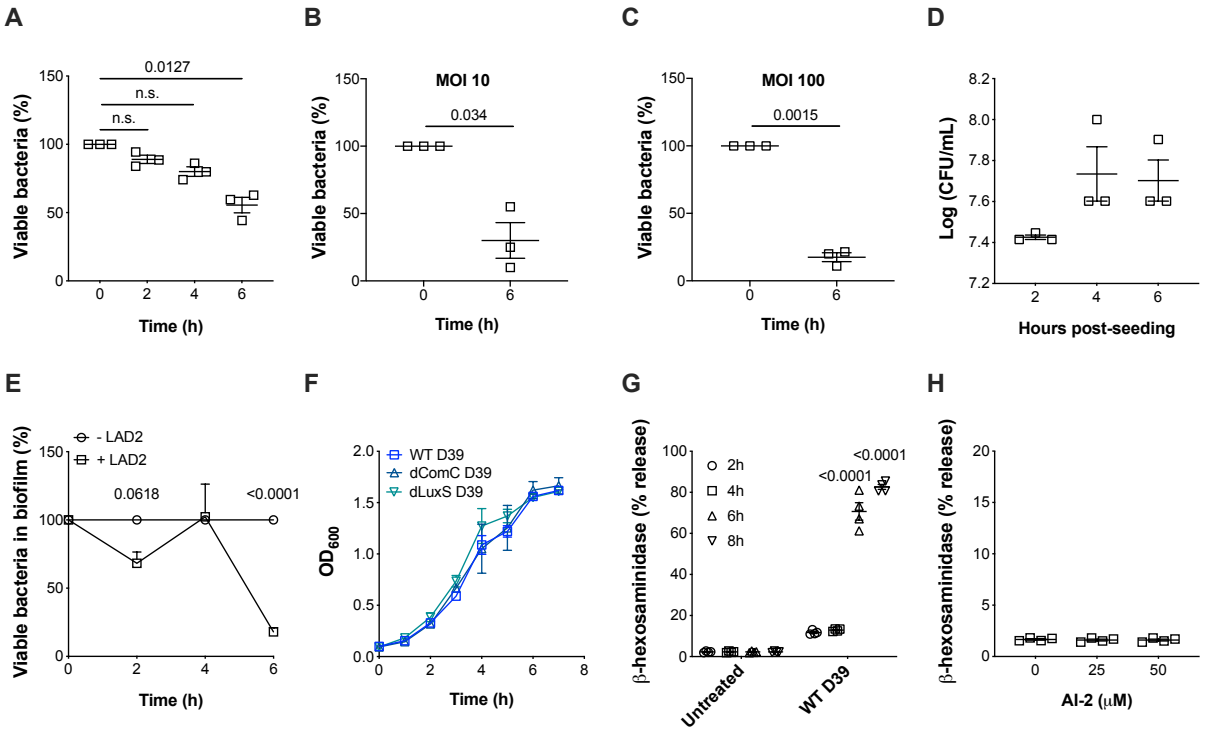

Figure S2. Antimicrobial activity of MRGPRX2-expressing mast cells *in vitro*, Related to Figure 2. (**A**) Time course analysis of the ability of LAD2 human mast cells to kill *Streptococcus pneumoniae* strain D39 in planktonic co-cultures (n=3). (**B, C**) The antibacterial activity of LAD2 cells was tested against two different multiplicities of infection (MOI) in planktonic co-cultures. MOI 10 means 10 D39 CFUs per mast cell. MOI 100 means 100 D39 CFUs per mast cell (n=3). (**D**) D39 can form a biofilm on the surface of a microtiter plate as early as 2 h post-seeding (n=3). (**E**) Inhibition of the growth of the biofilm by LAD2 cells is only evident at 6 h post-seeding (n=5). (**F**) Comparison of the three D39 strains in terms of their growth in liquid cultures (n=3). (**G**) Time course analysis of the β-hexosaminidase release from LAD2 cells in response to bacterial supernatants (n=4). (**H**) The release of β-hexosaminidase from LAD2 cells in response to autoinducer (AI)-2 (n=4). Mean ± SEM. Unpaired two-tailed Student's *t*-test.

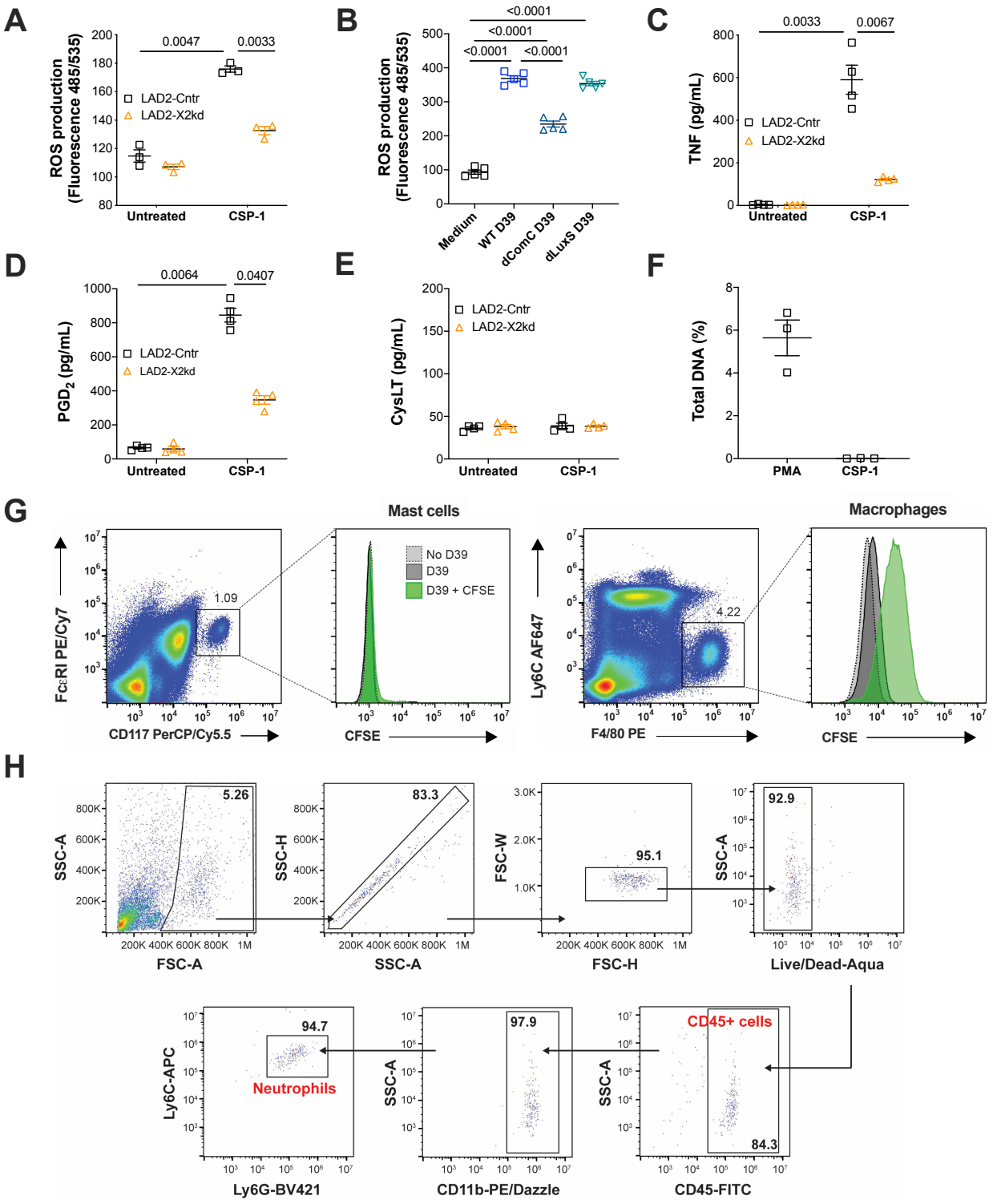

Figure S3. Mechanism of Mrgpr-mediated bacterial clearance, Related to Figure 3. (**A**) LAD2 cells transfected with either control siRNA (LAD2-Cntr) or MRGPRX2 siRNA (LAD2-X2kd) were treated with CSP-1 and cell-free supernatants were analyzed for ROS generation (n=3).

(**B**) LAD2 cells were incubated with bacterial supernatants and cell-free supernatants were analyzed for ROS generation (n=3). (**C-E**) LAD2-Cntr and LAD2-X2kd cells were treated with CSP-1 and cell-free supernatants were analyzed for (**C**) TNF, (**D**) PGD_2, and (**E**) CysLT production (n=4). (**F**) LAD2 cells were stimulated with PMA or CSP-1 and the total percentage of DNA released was quantified (n=3). (**G**) Phagocytosis of *S. pneumoniae* D39 was assessed by flow cytometry. CFSE-stained bacteria were intraperitoneally injected into WT mice and peritoneal lavage fluid was collected. Mast cells were gated as $CD117^{+}Fc\varepsilon RI^{+}$ and macrophages as $Ly6C^{-}F4/80^{+}$ (n=3). (**H**) Gating strategy for immune cells in nasal lavage fluid samples obtained from WT and $Mrgprb2^{MUT}$ animals colonized with D39. The cells were initially gated for single cells based on forward and side scatter (FSC-A/SSC-A) followed by two double exclusion gates (SSC-A/SSC-H and FSC-H/FSC-W). Then, dead cells were excluded based on their positivity for Live/Dead Fixable Aqua Dead cell staining. Live cells were then gated for $CD45^{+}$ cells. Neutrophils were identified as $CD11b^{+}Ly6G^{+}Ly6C^{-}$ cells. Mean $\pm$ SEM. Two-way ANOVA with Sidak's multiple comparison test.

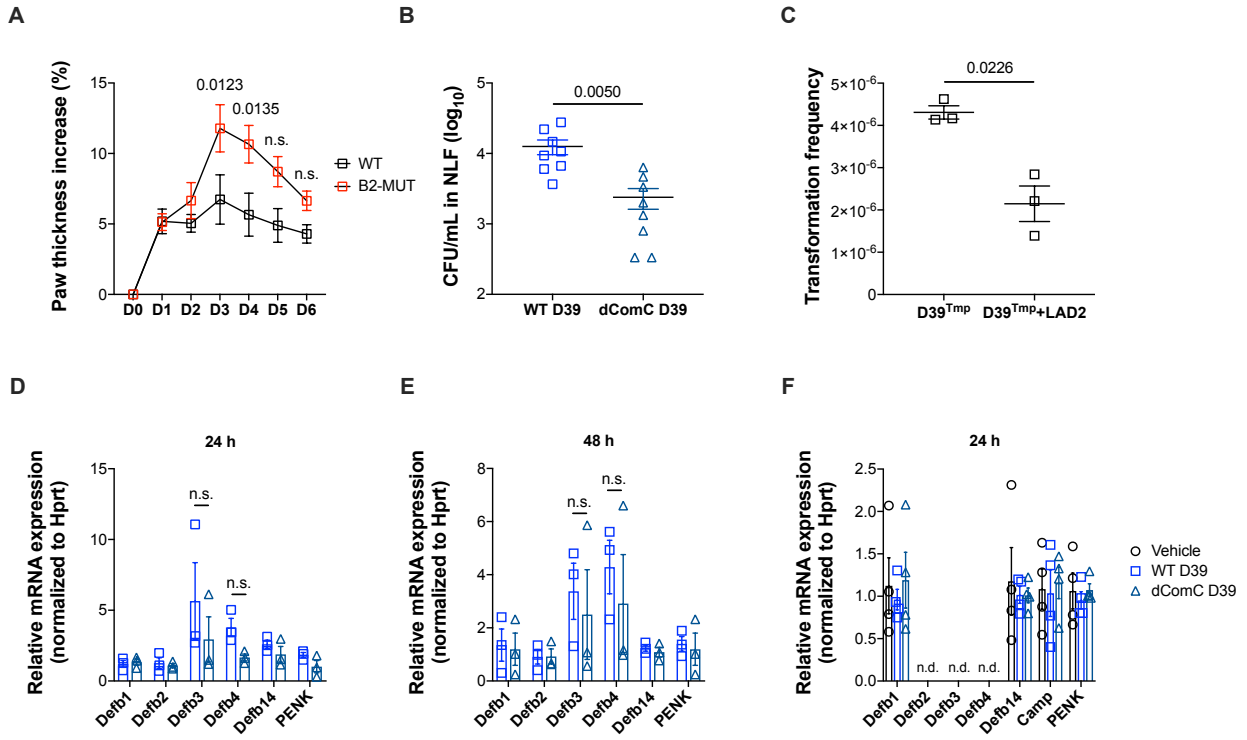

Figure S4. Effect of bacteria and associated quorum sensing on the production of endogenous cationic peptides, Related to Figure 4. (**A**) Paw edema following subcutaneous injection with *P. aeruginosa* in hind paws of WT and Mrgprb2^{MUT} mice (n=5). (**B**) Clearance of D39 from the nasopharynx of Mrgprb2^{MUT} mice after infection with WT and CSP-1-deficient dComC D39 (n=8). (**C**) Transformation frequency of D39 in the presence of absence of LAD2 cells (n=3). (**D, E**) Supernatants harvested from WT or dComC D39 were injected subcutaneously into the hind paws of mice. After 24 h (**D**) and 48 h (**E**), skin tissues were collected to measure the expression of mouse defensin (Def)b1, Defb2, Defb3, Dfeb4, Defb14, and proenkephalin (PENK; n=3). (**F**) Mice were intranasally colonized with WT D39, dComC D39 or vehicle. After 24 h, airway tissue was collected to measure the expression of Defb1, Defb2, Defb3, Dfeb4, Defb14, cathelicidin Camp, and PENK (n=4). Mean ± SEM. Unpaired two-tailed Student's *t*-test. n.s. = not significant. n.d. = not detected.

Table S1. List summarizing intracellular calcium (Ca^{2+}) responses to bacterial products by HEK293 cells transfected with MRGPRX2 and Mrgprb2. Positive (Ca^{2+} elevation) and negative (no change in Ca^{2+}) responses are indicated as 'checks' and 'crosses', respectively. QSM = quorum sensing molecule, Related to Figure 1.

Product	Bacteria	Net charge	X2	b2
Peptide QSM				
Competence-Stimulating Peptide; CSP-1	*Streptococcus pneumoniae*	+4.0	✓	✓
CSP-2	*Streptococcus pneumoniae*	+3.0	✓	✓
CSP (variant of CSP-1)	*Streptococcus pneumoniae*	+3.0	✓	✓
Bacteriocin Inducing Peptide; BIP1	*Streptococcus pneumoniae*	-0.9	✗	✗
BIP2	*Streptococcus pneumoniae*	-0.9	✗	✗
Entf-metabolite	*Enterococcus faecium*	+2.9	✓	✓
SilCR	*Streptococcus pyogenes*	+3.1	✓	✓
Streptin-1	*Streptococcus pyogenes*	+2.8	✓	✓
Competence and Sporulation Factor phrC1	*Bacillus subtilis*	+1.1	✗	✗
PaEDF3	*Pseudomonas aeruginosa*	+1.0	✗	✗
Non-peptide QSM				
N-3-oxo-dodecanoyl-L-Homoserine lactone; 3-HSL	*Pseudomonas aeruginosa*		✗	✗
N-butyryl-L-Homoserine lactone; HSL	*Pseudomonas aeruginosa*		✗	✗
4-hydroxy-2-heptylquinoline; HHQ	*Pseudomonas aeruginosa*		✗	✗
2,4-dihydroxyquinoline; DHQ	*Pseudomonas aeruginosa*		✗	✗
3,4-dihydroxy-2-heptylquinoline; PQS	*Pseudomonas aeruginosa*		✗	✗
Pyocyanin	*Pseudomonas aeruginosa*		✗	✗
Autoinducer-2	*Universal*		✗	✗
Non-QSM Peptide				
Delta toxin (1-26)	*Staphylococcus aureus*	+1.0	✗	✗
Staphylococcal Enterotoxin B Domain; SEB (144-153)	*Staphylococcus aureus*	+1.0	✗	✗
Listeriolysin O; LLO (91-99)	*Listeria monocytogenes*	-1.0	✗	✗
CFP10 (71-85)	*Mycobacterium tuberculosis*	+1.0	✗	✗
Bacteriocins				
Pediocin	*Pediococcus acidilactici*	+4.0	✓	✓
Nisin	*Lactococcus lactis*	+3.9	✓	✓
Gallidermin	*Staphylococcus gallinarum*	+3.0	✓	✓

Table S2. Sequences of quorum sensing peptides with positive activities on MRGPRX2 and Mrgprb2. QSM = quorum sensing molecule, Related to Figure 1.

Peptide QSM	Bacteria	Sequence
CSP-1	*Streptococcus pneumoniae*	*EMRLSKFFRDFILQRKK*
CSP-2	*Streptococcus pneumoniae*	*EMRISRIILDFLFLRKK*
CSP (variant of CSP-1)	*Streptococcus pneumoniae*	*ESRLPKILLDFLFLRKK*
Entf-metabolite	*Enterococcus faecium*	*AGTKPQGKPASNLVECVFSLFKKCN*
Streptin-1	*Streptococcus pyogenes*	*VGSRYLCTPGSCWKLVCFTTTVK*

Table S3. EC_{50} of quorum sensing peptides to activate MRGPRX2-HEK and Mrgprb2-HEK cells. The EC_{50} values were determined from dose response curves from three independent experiments, Related to Figure 1.

Peptide QSM	Net charge	EC_{50} for X2 (μM)	EC_{50} for b2 (μM)
CSP-1	+4.0	0.78 ± 0.05	28.22 ± 3.8
CSP-2	+3.0	4.95 ± 0.1	45.5 ± 0.36
CSP	+3.0	4.05 ± 0.38	48.43 ± 1.94
Entf	+2.9	9.19 ± 1.82	57.81 ± 14.9
Streptin-1	+2.8	9.25 ± 0.65	24.01 ± 0.74