HER3-targeted affibodies with optimized formats reduce ovarian cancer progression in a mouse xenograft model

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Supplemental file

Affibody construct protein and DNA sequences:

1. Monovalent HER3 affibody:

Protein:

AHHHHHHVVDNKFNKERYLAYYEIWQLPNLNRTQKAAFIGSLQDDPSQSANLLAEAKK

LNDAQAPK

DNA:

GCACATCACCACCACCATCACGTGGTCGATAACAAATTCAACAAAGAACGCTACTT

AGCATATTACGAGATTTGGCAGTTACCGAACCTGAACCGGACCCAAAAAGCTGCGT

TTATTGGCAGCTTGCAAGACGACCCGTCACAGAGCGCCAACCTGCTGGCCGAAGCG

AAGAAGCTGAATGATGCGCAAGCACCCAAA

2. Bivalent HER3 affibody (64 amino acid linker):

Protein:

AHHHHHHVVDNKFNKERYLAYYEIWQLPNLNRTQKAAFIGSLQDDPSQSANLLAEAKK

LNDAQAPKGGASGAGGSEGGGSEGGTSGATASGAGGSEGGGSEGGTSGATASGAGGSE

GGGSEGGTSGATGGVDNKFNKERYLAYYEIWQLPNLNRTQKAAFIGSLQDDPSQSANL

LAEAKKLNDAQAPK

DNA:

GCACATCACCACCACCATCACGTGGTCGATAACAAATTCAACAAAGAACGCTACTT

AGCATATTACGAGATTTGGCAGTTACCGAACCTGAACCGGACCCAAAAAGCTGCGT

TTATTGGCAGCTTGCAAGACGACCCGTCACAGAGCGCCAACCTGCTGGCCGAAGCG

AAGAAGCTGAATGATGCGCAAGCACCCAAAGGCGGTGCCTCGGGCGCAGGGGGTA

GCGAAGGCGGGGGCTCCGAAGGTGGCACCTCGGGAGCCACCGCATCAGGGGCAGG

GGGCAGCGAAGGCGGCGGTTCGGAAGGGGGTACGTCAGGCGCGACCGCATCTGGG

GCGGGTGGGAGCGAAGGCGGAGGGTCCGAGGGTGGCACATCAGGCGCTACCGGTG

GTGTAGACAACAAATTTAACAAAGAGCGTTACCTGGCCTATTACGAAATTTGGCAAC

TGCCGAATCTGAACCGGACGCAGAAAGCTGCTTTCATTGGCAGCCTGCAGGATGAC

CCAAGCCAGTCGGCGAATCTTCTGGCTGAAGCGAAAAAGCTCAACGATGCACAAGC

CCCTAAA

3. Bivalent HER3 affibody (GGG linker):

Protein:

AHHHHHHVVDNKFNKERYLAYYEIWQLPNLNRTQKAAFIGSLQDDPSQSANLLAEAKK

LNDAQAPKGGGVDNKFNKERYLAYYEIWQLPNLNRTQKAAFIGSLQDDPSQSANLLAE

AKKLNDAQAPK

DNA:

GCACATCACCACCACCATCACGTGGTCGATAACAAATTCAACAAAGAACGCTACTT

AGCATATTACGAGATTTGGCAGTTACCGAACCTGAACCGGACCCAAAAAGCTGCGT

TTATTGGCAGCTTGCAAGACGACCCGTCACAGAGCGCCAACCTGCTGGCCGAAGCG

AAGAAGCTGAATGATGCGCAAGCACCCAAAGGCGGTGGTGTAGACAACAAATTTAA

CAAAGAGCGTTACCTGGCCTATTACGAAATTTGGCAACTGCCGAATCTGAACCGGAC

GCAGAAAGCTGCTTTCATTGGCAGCCTGCAGGATGACCCAAGCCAGTCGGCGAATC

TTCTGGCTGAAGCGAAAAAGCTCAACGATGCACAAGCCCCTAAA

4. Hexavalent linear HER3 affibody

Protein:

AHHHHHHVVDNKFNKERYLAYYEIWQLPNLNRTQKAAFIGSLQDDPSQSANLLAEAKK

LNDAQAPKGGGVDNKFNKERYLAYYEIWQLPNLNRTQKAAFIGSLQDDPSQSANLLAE

AKKLNDAQAPKGGGVDNKFNKERYLAYYEIWQLPNLNRTQKAAFIGSLQDDPSQSANL

LAEAKKLNDAQAPKGGGVDNKFNKERYLAYYEIWQLPNLNRTQKAAFIGSLQDDPSQS

ANLLAEAKKLNDAQAPKGGGVDNKFNKERYLAYYEIWQLPNLNRTQKAAFIGSLQDDP

SQSANLLAEAKKLNDAQAPKGGGVDNKFNKERYLAYYEIWQLPNLNRTQKAAFIGSLQ

DDPSQSANLLAEAKKLNDAQAPK

DNA:

GCACATCACCACCACCATCACGTGGTCGATAACAAATTCAACAAAGAACGCTACTT

AGCATATTACGAGATTTGGCAGTTACCGAACCTGAACCGGACCCAAAAAGCTGCGT

TTATTGGCAGCTTGCAAGACGACCCGTCACAGAGCGCCAACCTGCTGGCCGAAGCG

AAGAAGCTGAATGATGCGCAAGCACCCAAAGGCGGTGGTGTAGACAACAAATTTAA

CAAAGAGCGTTACCTGGCCTATTACGAAATTTGGCAACTGCCGAATCTGAACCGGAC

GCAGAAAGCTGCTTTCATTGGCAGCCTGCAGGATGACCCAAGCCAGTCGGCGAATC

TTCTGGCTGAAGCGAAAAAGCTCAACGATGCACAAGCCCCTAAAGGCGGTGGTGTC

GATAACAAATTCAACAAAGAACGCTACTTAGCATATTACGAGATTTGGCAGTTACCG

AACCTGAACCGGACCCAAAAAGCTGCGTTTATTGGCAGCTTGCAAGACGACCCGTC

ACAGAGCGCCAACCTGCTGGCCGAAGCGAAGAAGCTGAATGATGCGCAAGCACCCA

AAGGCGGTGGTGTAGACAACAAATTTAACAAAGAGCGTTACCTGGCCTATTACGAA

ATTTGGCAACTGCCGAATCTGAACCGGACGCAGAAAGCTGCTTTCATTGGCAGCCTG

CAGGATGACCCAAGCCAGTCGGCGAATCTTCTGGCTGAAGCGAAAAAGCTCAACGA

TGCACAAGCCCCTAAAGGCGGTGGTGTCGATAACAAATTCAACAAAGAACGCTACT

TAGCATATTACGAGATTTGGCAGTTACCGAACCTGAACCGGACCCAAAAAGCTGCG

TTTATTGGCAGCTTGCAAGACGACCCGTCACAGAGCGCCAACCTGCTGGCCGAAGC

GAAGAAGCTGAATGATGCGCAAGCACCCAAAGGCGGTGGTGTAGACAACAAATTTA

ACAAAGAGCGTTACCTGGCCTATTACGAAATTTGGCAACTGCCGAATCTGAACCGG

ACGCAGAAAGCTGCTTTCATTGGCAGCCTGCAGGATGACCCAAGCCAGTCGGCGAA

TCTTCTGGCTGAAGCGAAAAAGCTCAACGATGCACAAGCCCCTAAA

5. Monovalent EGFR affibody:

Protein:

AHHHHHHGGVDNKFNKEMWAAWEEIRNLPNLNGWQMTAFIASLVDDPSQSANLLAE

AKKLNDAQAPK

DNA:

GCACATCACCACCACCATCACGGTGGTGTGGATAACAAATTTAACAAAGAGATGTG

GGCGGCATGGGAAGAGATCCGTAATTTGCCTAACCTGAATGGCTGGCAAATGACCG

CGTTTATCGCTAGTTTGGTTGATGACCCGAGTCAATCGGCGAACTTACTTGCAGAGG

CGAAGAAATTAAACGACGCGCAGGCTCCGAAA

6. Bispecific HER3-EGFR bivalent affibody (64 AA linker):

Protein:

AHHHHHHVVDNKFNKERYLAYYEIWQLPNLNRTQKAAFIGSLQDDPSQSANLLAEAKK

LNDAQAPKGGASGAGGSEGGGSEGGTSGATASGAGGSEGGGSEGGTSGATASGAGGSE

GGGSEGGTSGATGGVDNKFNKEMWAAWEEIRNLPNLNGWQMTAFIASLVDDPSQSAN

LLAEAKKLNDAQAPK

DNA:

GCACATCACCACCACCATCACGTGGTCGATAACAAATTCAACAAAGAACGCTACTT
AGCATATTACGAGATTTGGCAGTTACCGAACCTGAACCGGACCCAAAAAGCTGCGT
TTATTGGCAGCTTGCAAGACGACCCGTCACAGAGCGCCAACCTGCTGGCCGAAGCG
AAGAAGCTGAATGATGCGCAAGCACCCAAAGGCGGTGCCTCGGGCGCAGGGGGTA
GCGAAGGCGGGGGCTCCGAAGGTGGCACCTCGGGAGCCACCGCATCAGGGGCAGG
GGGCAGCGAAGGCGGCGGTTCGGAAGGGGGTACGTCAGGCGCGACCGCATCTGGG
GCGGGTGGGAGCGAAGGCGGAGGGTCCGAGGGTGGCACATCAGGCGCTACCGGTG
GTGTGGATAACAAATTTAACAAAGAGATGTGGGCGGCATGGGAAGAGATCCGTAAT
TTGCCTAACCTGAATGGCTGGCAAATGACCGCGTTTATCGCTAGTTTGGTTGATGAC
CCGAGTCAATCGGCGAACTTACTTGCAGAGGCGAAGAAATTAAACGACGCGCAGGC
TCCGAAA

7. Bispecific HER3-EGFR bivalent affibody (GGG linker):

Protein:

AHHHHHHVVDNKFNKERYLAYYEIWQLPNLNRTQKAAFIGSLQDDPSQSANLLAEAKK
LNDAQAPKGGGVDNKFNKEMWAAWEEIRNLPNLNGWQMTAFIASLVDDPSQSANLLA
EAKKLNDAQAPK

DNA:

GCACATCACCACCACCATCACGTGGTCGATAACAAATTCAACAAAGAACGCTACTT
AGCATATTACGAGATTTGGCAGTTACCGAACCTGAACCGGACCCAAAAAGCTGCGT
TTATTGGCAGCTTGCAAGACGACCCGTCACAGAGCGCCAACCTGCTGGCCGAAGCG
AAGAAGCTGAATGATGCGCAAGCACCCAAAGGCGGTGGTGTGGATAACAAATTTAA
CAAAGAGATGTGGGCGGCATGGGAAGAGATCCGTAATTTGCCTAACCTGAATGGCT

GGCAAATGACCGCGTTTATCGCTAGTTTGGTTGATGACCCGAGTCAATCGGCGAACT

TACTTGCAGAGGCGAAGAAATTAAACGACGCGCAGGCTCCGAAA

8. Bivalent EGFR affibody (64 AA linker):

Protein:

AHHHHHHVVDNKFNKEMWAAWEEIRNLPNLNGWQMTAFIASLVDDPSQSANLLAEA

KKLNDAQAPKGGASGAGGSEGGGSEGGTSGATASGAGGSEGGGSEGGTSGATASGAG

GSEGGGSEGGTSGATGGVDNKFNKEMWAAWEEIRNLPNLNGWQMTAFIASLVDDPSQ

SANLLAEAKKL

DNA:

GCACATCACCACCACCATCACGTGGTGGATAACAAATTTAACAAAGAGATGTGGGC

GGCATGGGAAGAGATCCGTAATTTGCCTAACCTGAATGGCTGGCAAATGACCGCGT

TTATCGCTAGTTTGGTTGATGACCCGAGTCAATCGGCGAACTTACTTGCAGAGGCGA

AGAAATTAAACGACGCGCAGGCTCCGAAAGGCGGTGCCTCGGGCGCAGGGGGTAG

CGAAGGCGGGGGCTCCGAAGGTGGCACCTCGGGAGCCACCGCATCAGGGGCAGGG

GGCAGCGAAGGCGGCGGTTCGGAAGGGGGTACGTCAGGCGCGACCGCATCTGGGG

CGGGTGGGAGCGAAGGCGGAGGGTCCGAGGGTGGCACATCAGGCGCTACCGGTGGT

GTGGATAACAAATTTAACAAAGAGATGTGGGCGGCATGGGAAGAGATCCGTAATTT

GCCTAACCTGAATGGCTGGCAAATGACCGCGTTTATCGCTAGTTTGGTTGATGACCC

GAGTCAATCGGCGAACTTACTTGCAGAGGCGAAGAAATTAAA

9. Bivalent EGFR affibody (GGG linker):

Protein:

AHHHHHHVVDNKFNKEMWAAWEEIRNLPNLNGWQMTAFIASLVDDPSQSANLLAEA

KKLNDAQAPKGGGVDNKFNKEMWAAWEEIRNLPNLNGWQMTAFIASLVDDPSQSANL

LAEAKKLNDAQAPK

DNA:

GGCACATCACCACCACCATCACGTGGTGGATAACAAATTTAACAAAGAGATGTGGG

CGGCATGGGAAGAGATCCGTAATTTGCCTAACCTGAATGGCTGGCAAATGACCGCG

TTTATCGCTAGTTTGGTTGATGACCCGAGTCAATCGGCGAACTTACTTGCAGAGGCG

AAGAAATTAAACGACGCGCAGGCTCCGAAAGGCGGTGGTGTGGATAACAAATTTAA

CAAAGAGATGTGGGCGGCATGGGAAGAGATCCGTAATTTGCCTAACCTGAATGGCT

GGCAAATGACCGCGTTTATCGCTAGTTTGGTTGATGACCCGAGTCAATCGGCGAACT

TACTTGCAGAGGCGAAGAAATTAAACGACGCGCAGGCTCCGAAA

10. Bivalent affibody HER3-Zwt (64 AA linker)

Protein:

AHHHHHHVVDNKFNKERYLAYYEIWQLPNLNRTQKAAFIGSLQDDPSQSANLLAEAKK

LNDAQAPKGGASGAGGSEGGGSEGGTSGATASGAGGSEGGGSEGGTSGATASGAGGSE

GGGSEGGTSGATGGVDNKFNKEQQNAFYEILHLPNLNEEQRNAFIQSLKDDPSQSANLL

AEAKKLNDAQAPK

DNA:

GCACATCACCACCACCATCACGTGGTCGATAACAAATTCAACAAAGAACGCTACTT

AGCATATTACGAGATTTGGCAGTTACCGAACCTGAACCGGACCCAAAAAGCTGCGT

TTATTGGCAGCTTGCAAGACGACCCGTCACAGAGCGCCAACCTGCTGGCCGAAGCG

AAGAAGCTGAATGATGCGCAAGCACCCAAAGGCGGTGCCTCGGGCGCAGGGGGTA

GCGAAGGCGGGGGCTCCGAAGGTGGCACCTCGGGAGCCACCGCATCAGGGGCAGG

GGGCAGCGAAGGCGGCGGTTCGGAAGGGGGTACGTCAGGCGCGACCGCATCTGGG
GCGGGTGGGAGCGAAGGCGGAGGGTCCGAGGGTGGCACATCAGGCGCTACCGGTG
GTGTAGACAACAAATTTAACAAAGAGCAGCAGAACGCTTTCTATGAAATACTGCAC
TTACCGAACCTGAACGAGGAGCAGCGGAATGCTTTTATACAGTCGTTGAAGGATGA
CCCAAGCCAGTCGGCGAATCTTCTGGCTGAAGCGAAAAAGCTCAACGATGCACAAG
CCCCTAAA

11. Monovalent HER3-ABD affibody

Protein:

AHHHHHHVVDNKFNKERYLAYYEIWQLPNLNRTQKAAFIGSLQDDPSQSANLLAEAKK
LNDAQAPKGGGLAEAKVLANRELDKYGVSDFYKRLINKAKTVEGVEALKLHILAALP

DNA:

GCACATCACCACCACCATCACGTGGTCGATAACAAATTCAACAAAGAACGCTACTT
AGCATATTACGAGATTTGGCAGTTACCGAACCTGAACCGGACCCAAAAAGCTGCGT
TTATTGGCAGCTTGCAAGACGACCCGTCACAGAGCGCCAACCTGCTGGCCGAAGCG
AAGAAGCTGAATGATGCGCAAGCACCCAAAGGAGGGGGACTTGCAGAAGCCAAAG
TCTTAGCGAATAGAGAACTGGATAAATATGGCGTGTCCGACTTCTATAAGCGTCTGA
TAAATAAAGCGAAGACCGTTGAGGGAGTTGAAGCCCTGAAGTTGCATATTTTGGCT
GCCCTTCCA

12. Monovalent ABD-HER3 affibody

Protein:

AHHHHHHGGGLAEAKVLANRELDKYGVSDFYKRLINKAKTVEGVEALKLHILAALPG

GGVDNKFNKERYLAYYEIWQLPNLNRTQKAAFIGSLQDDPSQSANLLAEAKKLNDAQA

PK

DNA:

GCACATCACCACCACCATCACGGCGGTGGTCTTGCAGAAGCCAAAGTCTTAGCGAA

TAGAGAACTGGATAAATATGGCGTGTCCGACTTCTATAAGCGTCTGATAAATAAAGC

GAAGACCGTTGAGGGAGTTGAAGCCCTGAAGTTGCATATTTTGGCTGCCCTTCCAGG

AGGGGGAGTCGATAACAAATTCAACAAAGAACGCTACTTAGCATATTACGAGATTT

GGCAGTTACCGAACCTGAACCGGACCCAAAAAGCTGCGTTTATTGGCAGCTTGCAA

GACGACCCGTCACAGAGCGCCAACCTGCTGGCCGAAGCGAAGAAGCTGAATGATGC

GCAAGCACCCAAA

13. Bivalent HER3-HER3-ABD

Protein:

AHHHHHHVVDNKFNKERYLAYYEIWQLPNLNRTQKAAFIGSLQDDPSQSANLLAEAKK

LNDAQAPKGGGVDNKFNKERYLAYYEIWQLPNLNRTQKAAFIGSLQDDPSQSANLLAE

AKKLNDAQAPKGGGLAEAKVLANRELDKYGVSDFYKRLINKAKTVEGVEALKLHILA

ALP

DNA:

GCACATCACCACCACCATCACGTGGTCGATAACAAATTCAACAAAGAACGCTACTT

AGCATATTACGAGATTTGGCAGTTACCGAACCTGAACCGGACCCAAAAAGCTGCGT

TTATTGGCAGCTTGCAAGACGACCCGTCACAGAGCGCCAACCTGCTGGCCGAAGCG

AAGAAGCTGAATGATGCGCAAGCACCCAAAGGCGGTGGTGTAGACAACAAATTTAA

CAAAGAGCGTTACCTGGCCTATTACGAAATTTGGCAACTGCCGAATCTGAACCGGAC

GCAGAAAGCTGCTTTCATTGGCAGCCTGCAGGATGACCCAAGCCAGTCGGCGAATC

TTCTGGCTGAAGCGAAAAAGCTCAACGATGCACAAGCCCCTAAAGGAGGGGGACTT

GCAGAAGCCAAAGTCTTAGCGAATAGAGAACTGGATAAATATGGCGTGTCCGACTT

CTATAAGCGTCTGATAAATAAAGCGAAGACCGTTGAGGGAGTTGAAGCCCTGAAGT

TGCATATTTTGGCTGCCCTTCCA

14. Bivalent ABD-HER3-HER3

Protein:

AHHHHHHGGGLAEAKVLANRELDKYGVSDFYKRLINKAKTVEGVEALKLHILAALPG

GGVDNKFNKERYLAYYEIWQLPNLNRTQKAAFIGSLQDDPSQSANLLAEAKKLNDAQA

PKGGGVDNKFNKERYLAYYEIWQLPNLNRTQKAAFIGSLQDDPSQSANLLAEAKKLND

AQAPK

DNA:

GCACATCACCACCACCATCACGGCGGTGGTCTTGCAGAAGCCAAAGTCTTAGCGAA

TAGAGAACTGGATAAATATGGCGTGTCCGACTTCTATAAGCGTCTGATAAATAAAGC

GAAGACCGTTGAGGGAGTTGAAGCCCTGAAGTTGCATATTTTGGCTGCCCTTCCAGG

AGGGGGAGTCGATAACAAATTCAACAAAGAACGCTACTTAGCATATTACGAGATTT

GGCAGTTACCGAACCTGAACCGGACCCAAAAAGCTGCGTTTATTGGCAGCTTGCAA

GACGACCCGTCACAGAGCGCCAACCTGCTGGCCGAAGCGAAGAAGCTGAATGATGC

GCAAGCACCCAAAGGCGGTGGTGTAGACAACAAATTTAACAAAGAGCGTTACCTGG

CCTATTACGAAATTTGGCAACTGCCGAATCTGAACCGGACGCAGAAAGCTGCTTTCA

TTGGCAGCCTGCAGGATGACCCAAGCCAGTCGGCGAATCTTCTGGCTGAAGCGAAA

AAGCTCAACGATGCACAAGCCCCTAAA

15. Bivalent HER3-ABD-HER3

Protein:

AHHHHHHVVDNKFNKERYLAYYEIWQLPNLNRTQKAAFIGSLQDDPSQSANLLAEAKK

LNDAQAPKGGGLAEAKVLANRELDKYGVSDFYKRLINKAKTVEGVEALKLHILAALPG

GGVDNKFNKERYLAYYEIWQLPNLNRTQKAAFIGSLQDDPSQSANLLAEAKKLNDAQA

PK

DNA:

GCACATCACCACCACCATCACGTGGTCGATAACAAATTCAACAAAGAACGCTACTT

AGCATATTACGAGATTTGGCAGTTACCGAACCTGAACCGGACCCAAAAAGCTGCGT

TTATTGGCAGCTTGCAAGACGACCCGTCACAGAGCGCCAACCTGCTGGCCGAAGCG

AAGAAGCTGAATGATGCGCAAGCACCCAAAGGCGGTGGTCTTGCAGAAGCCAAAGT

CTTAGCGAATAGAGAACTGGATAAATATGGCGTGTCCGACTTCTATAAGCGTCTGAT

AAATAAAGCGAAGACCGTTGAGGGAGTTGAAGCCCTGAAGTTGCATATTTTGGCTG

CCCTTCCAGGAGGGGGAGTAGACAACAAATTTAACAAAGAGCGTTACCTGGCCTAT

TACGAAATTTGGCAACTGCCGAATCTGAACCGGACGCAGAAAGCTGCTTTCATTGGC

AGCCTGCAGGATGACCCAAGCCAGTCGGCGAATCTTCTGGCTGAAGCGAAAAAGCT

CAACGATGCACAAGCCCCTAAA

Figure legends

Figure S1: Immunoblot quantification of Figure 1. Quantified A. pHER3 B. pAkt and C. HER3 values are displayed for Figure 1. Blots were quantified using Odyssey CLx image system (Licor) Analysis Tool and normalized to β-actin. Results shown are mean +/- SEM of three independent experiments.

Figure S2: Immunoblot quantification of Figure 2. Quantified A. pHER3 B. pAkt and C. HER3 values are displayed for Figure 2. Blots were quantified using Odyssey CLx image system (Licor) Analysis Tool and normalized to β-actin. Results shown are mean +/- SEM of three independent experiments.

Figure S3: Immunoblot quantification of Figure 3. Quantified A. HER3 and B. EGFR values are displayed for Figure 3. Blots were quantified using Odyssey CLx image system (Licor) Analysis Tool and normalized to β-actin. Results shown are mean +/- SEM of three independent experiments.

Figure S4: Immunoblot quantification of Figure 4 (pHER3 and pAkt). Quantified A., B. pHER3 and B., C. pAkt values are displayed for Figure 4. Blots were quantified using Odyssey CLx image system (Licor) Analysis Tool and normalized to β-actin. Results shown are mean +/- SEM of three independent experiments.

Figure S5: Immunoblot quantification of Figure 4 (HER3). Quantified A, B. HER3 values are displayed for Figure 4. Blots were quantified using Odyssey CLx image system (Licor) Analysis

Tool and normalized to β-actin. Results shown are mean +/- SEM of three independent experiments.

Figure S6: Raw blots for Figure 1. Full length blots for Figure 1B, 1C (multiple exposures), and 1D are shown.

Figure S7: Raw blots for Figure 2. Full length blots for Figure 2B, 2C (multiple exposures), and 2D are shown.

Figure S8: Raw blots for Figure 3. Full length blots for Figure 3B, 3C, and 3D are shown.

Figure S9: Raw blots for Figure 4. Full length blots for Figure 4B, 4C (multiple exposures), 4D, and 4E are shown.

Figure S1

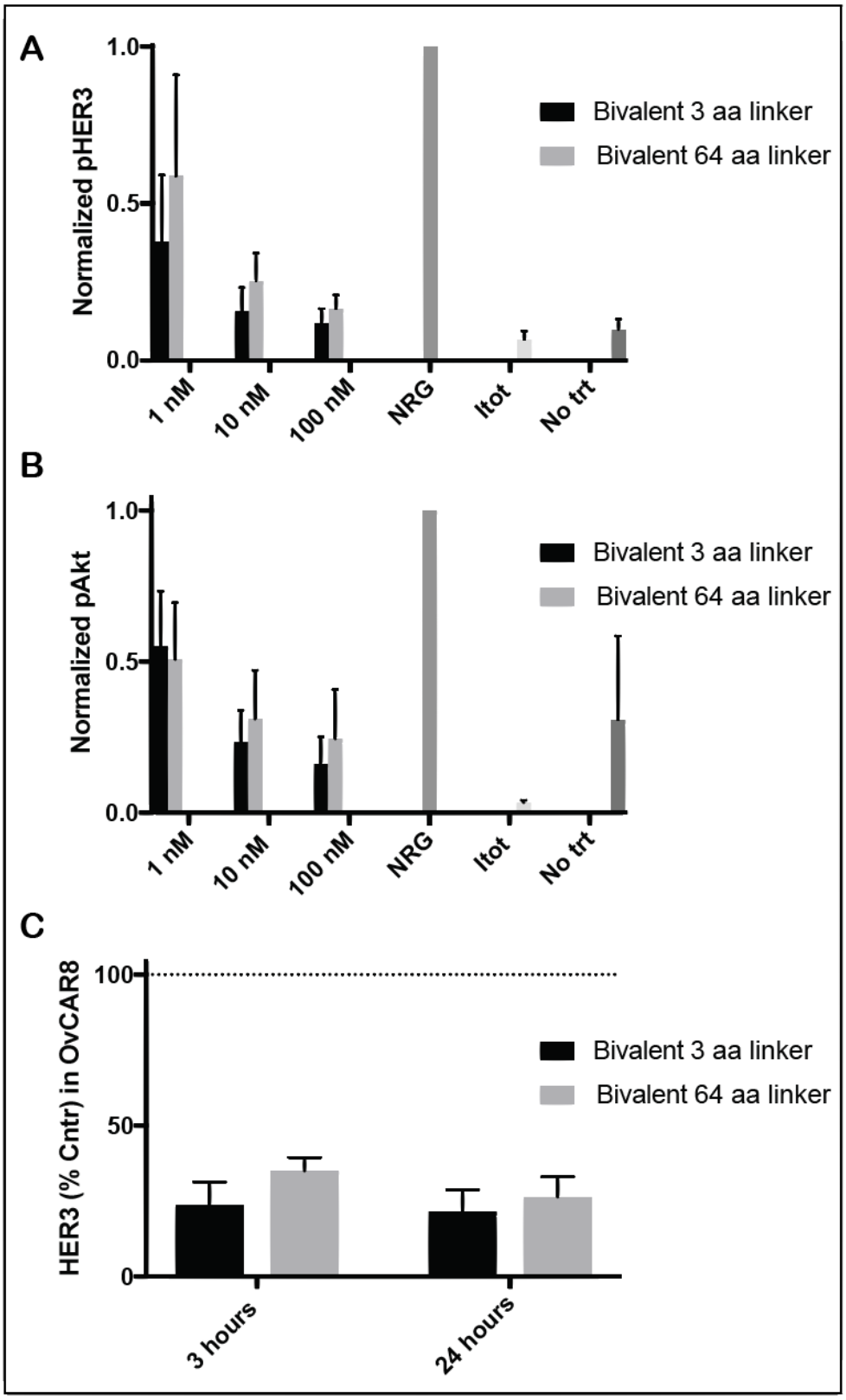
A
Normalized pHER3
1.0
0.5
0.0
Bivalent 3 aa linker
Bivalent 64 aa linker
1 nM
10 nM
100 nM
NRG
Itot
No trt
B
Normalized pAkt
1.0
0.5
0.0
Bivalent 3 aa linker
Bivalent 64 aa linker
1 nM
10 nM
100 nM
NRG
Itot
No trt
C
HER3 (% Cntr) in OvCAR8
100
50
0
Bivalent 3 aa linker
Bivalent 64 aa linker
3 hours
24 hours

Figure S2

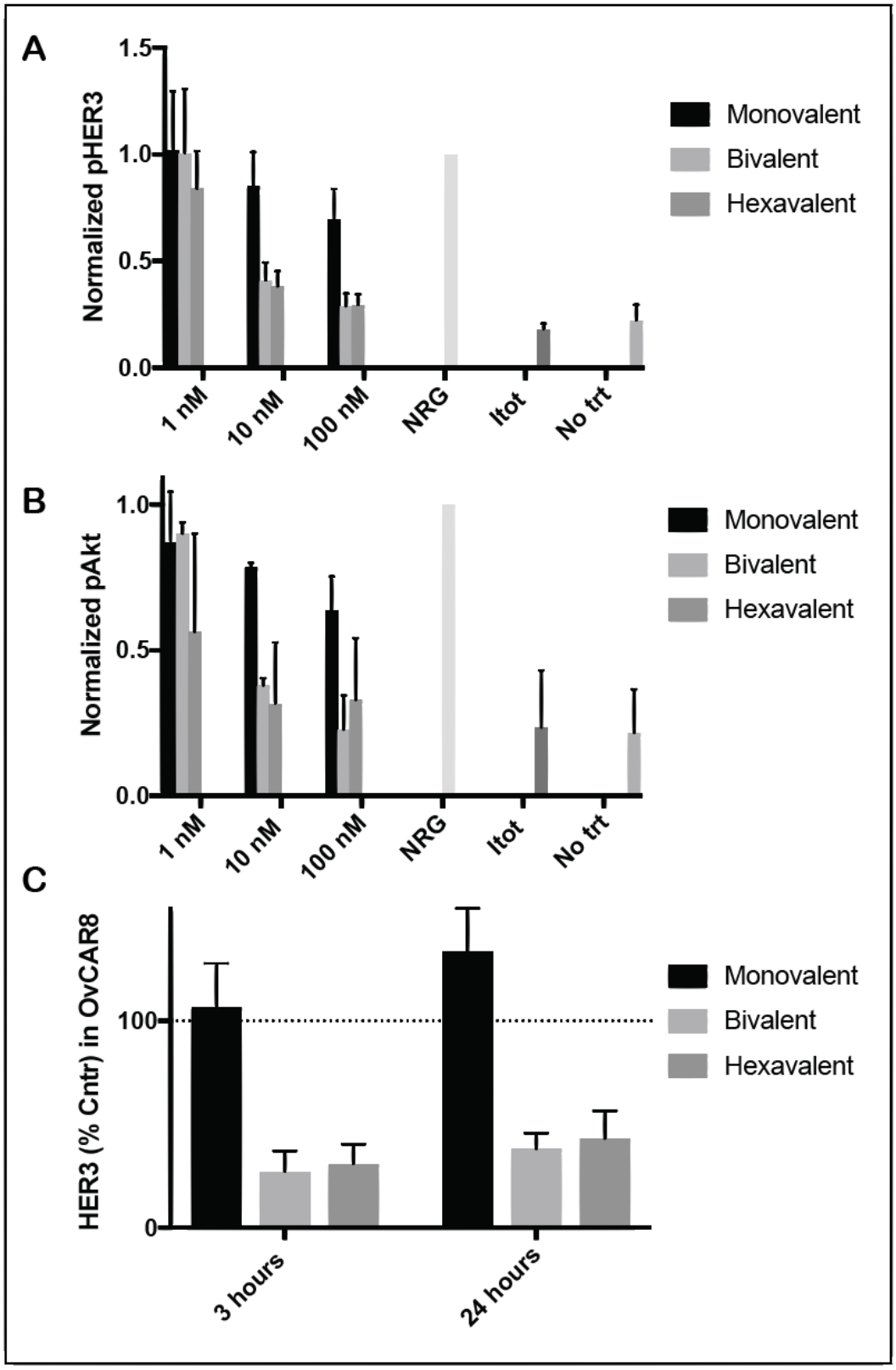
A
Normalized pHER3
1.5
1.0
0.5
0.0
1 nM
10 nM
100 nM
NRG
Itot
No trt
Monovalent
Bivalent
Hexavalent
B
Normalized pAkt
1.0
0.5
0.0
1 nM
10 nM
100 nM
NRG
Itot
No trt
Monovalent
Bivalent
Hexavalent
C
HER3 (% Cntr) in OvCAR8
100
0
3 hours
24 hours
Monovalent
Bivalent
Hexavalent

Figure S3

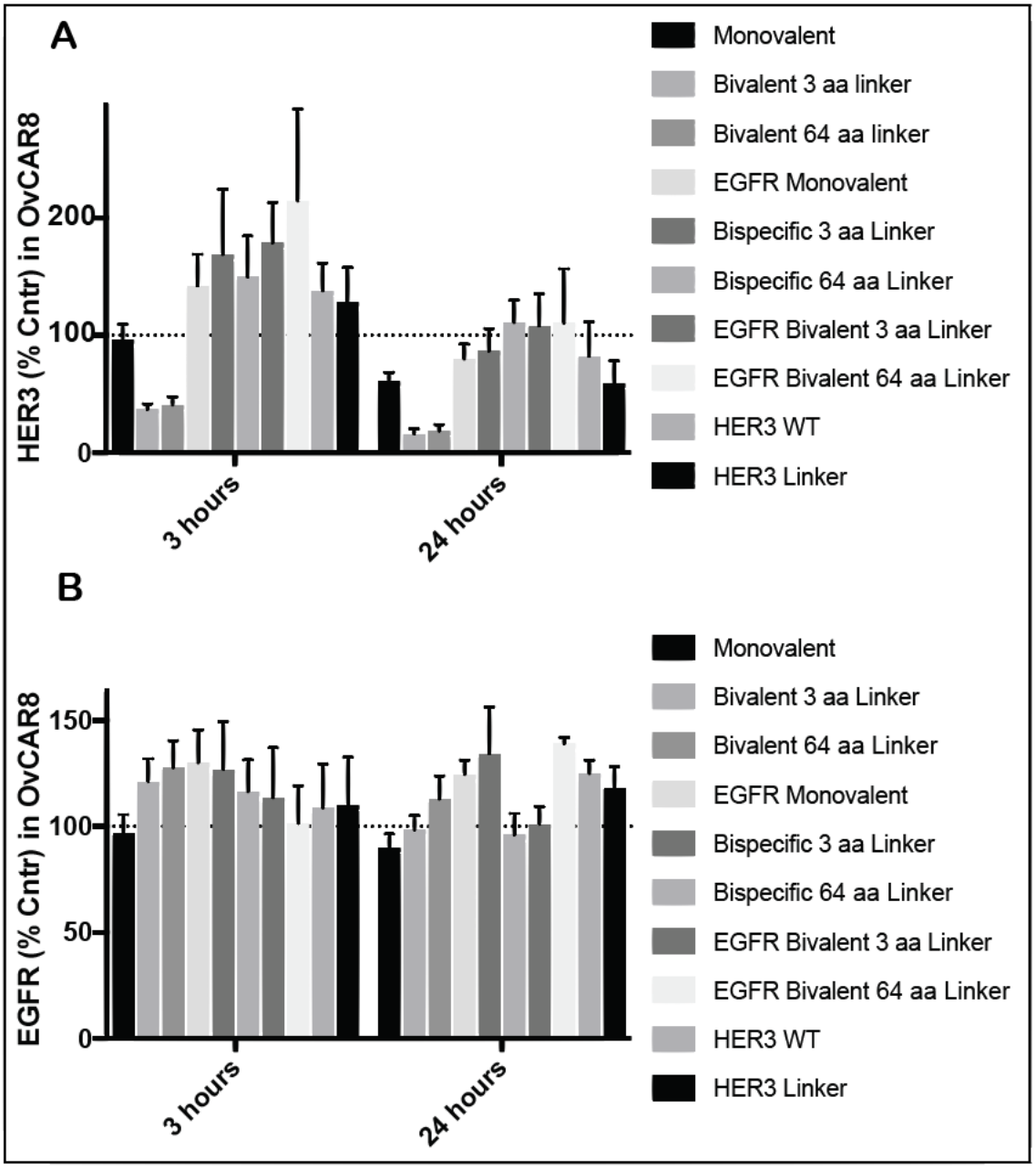
A
HER3 (% Cntr) in OvCAR8
200
100
0
3 hours
24 hours
Monovalent
Bivalent 3 aa linker
Bivalent 64 aa linker
EGFR Monovalent
Bispecific 3 aa Linker
Bispecific 64 aa Linker
EGFR Bivalent 3 aa Linker
EGFR Bivalent 64 aa Linker
HER3 WT
HER3 Linker
B
EGFR (% Cntr) in OvCAR8
150
100
50
0
3 hours
24 hours
Monovalent
Bivalent 3 aa Linker
Bivalent 64 aa Linker
EGFR Monovalent
Bispecific 3 aa Linker
Bispecific 64 aa Linker
EGFR Bivalent 3 aa Linker
EGFR Bivalent 64 aa Linker
HER3 WT
HER3 Linker

Figure S4

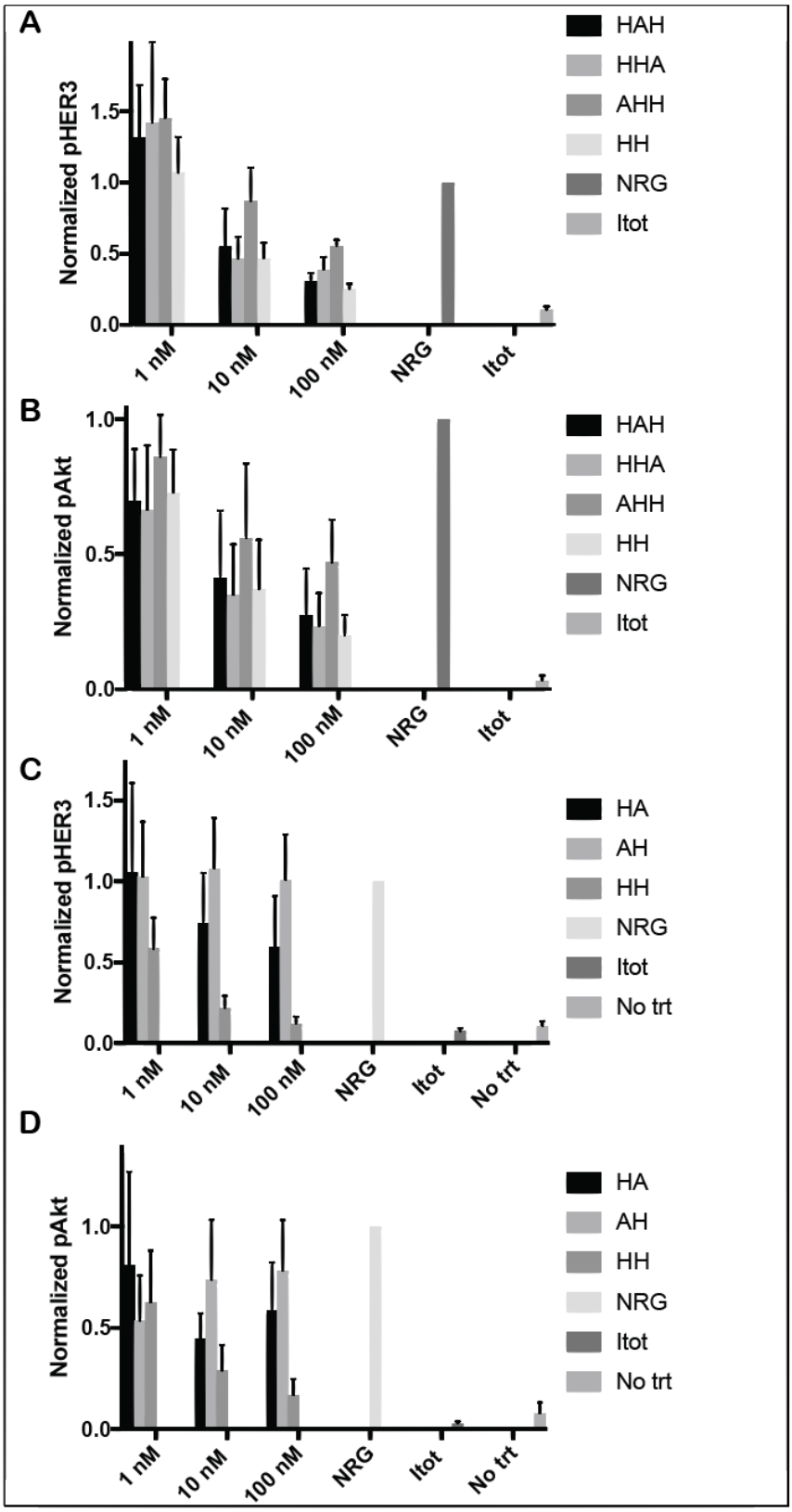
A
Normalized pHER3
1.5
1.0
0.5
0.0
1 nM
10 nM
100 nM
NRG
Itot
HAH
HHA
AHH
HH
NRG
Itot
B
Normalized pAkt
1.0
0.5
0.0
1 nM
10 nM
100 nM
NRG
Itot
HAH
HHA
AHH
HH
NRG
Itot
C
Normalized pHER3
1.5
1.0
0.5
0.0
1 nM
10 nM
100 nM
NRG
Itot
No trt
HA
AH
HH
NRG
Itot
No trt
D
Normalized pAkt
1.0
0.5
0.0
1 nM
10 nM
100 nM
NRG
Itot
No trt
HA
AH
HH
NRG
Itot
No trt

Figure S5

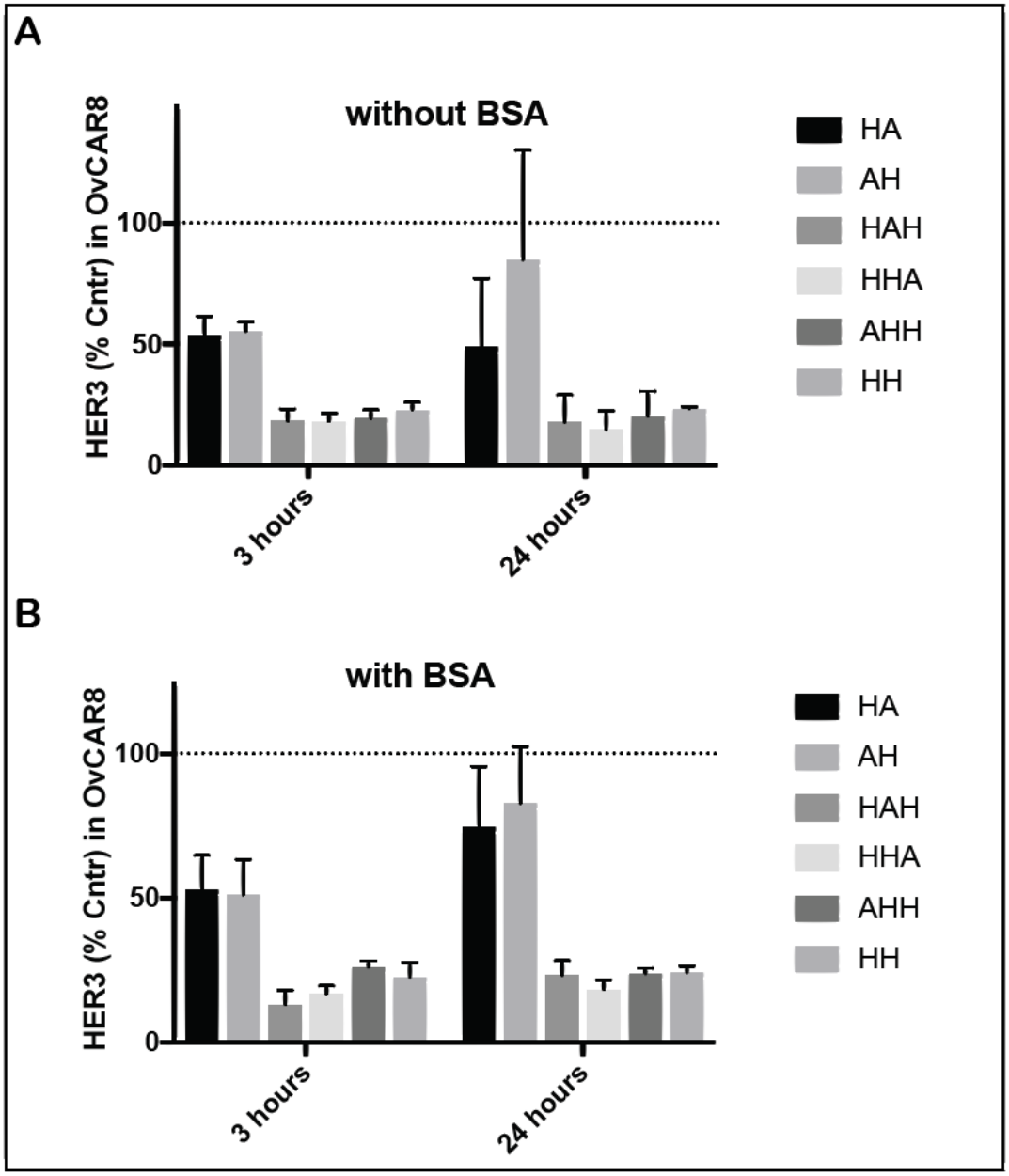
A
without BSA
HER3 (% Cntr) in OvCAR8
100
50
0
3 hours
24 hours
HA
AH
HAH
HHA
AHH
HH
B
with BSA
HER3 (% Cntr) in OvCAR8
100
50
0
3 hours
24 hours
HA
AH
HAH
HHA
AHH
HH

Figure S6

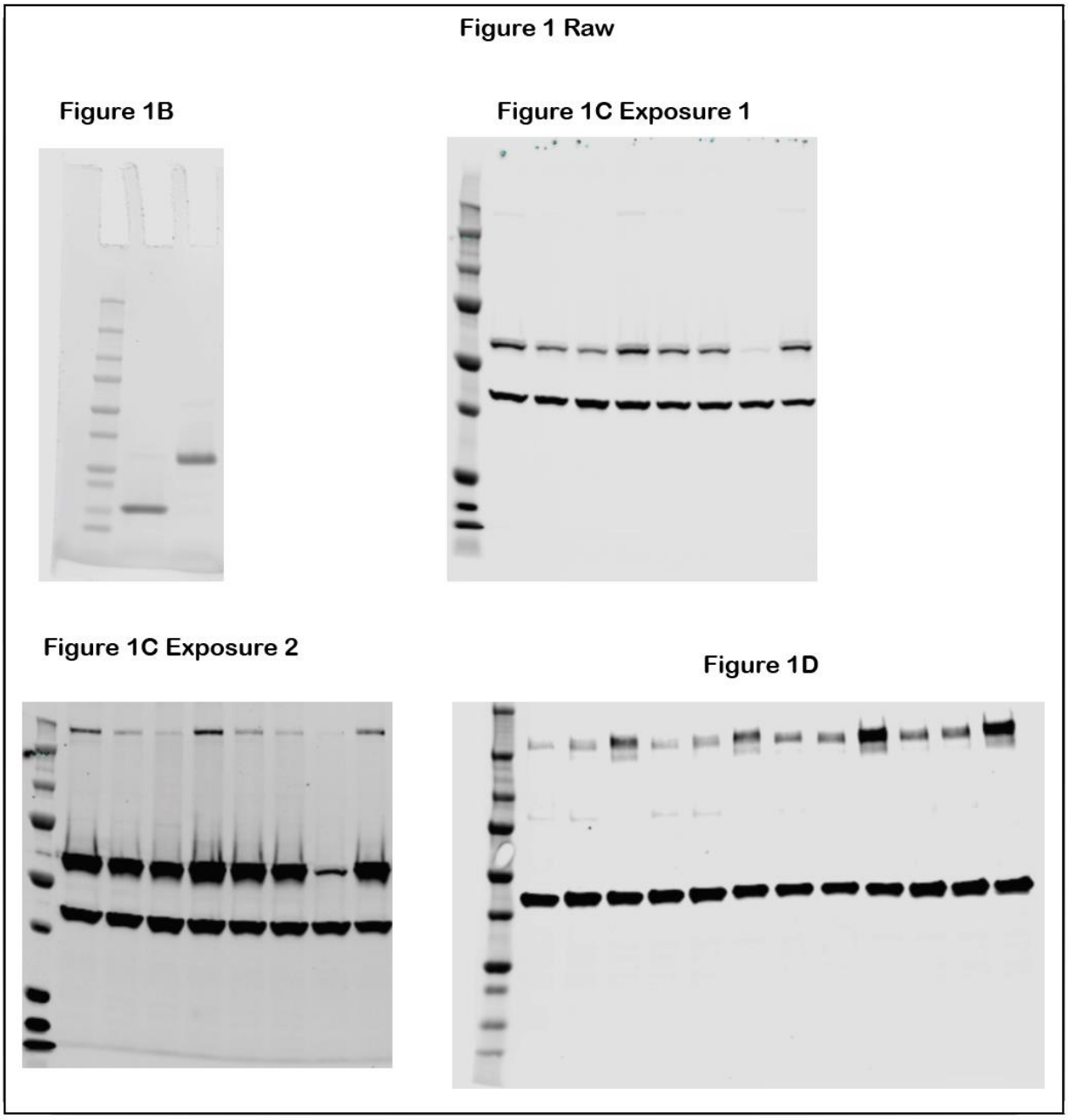
Figure 1 Raw
Figure 1B
Figure 1C Exposure 1
Figure 1C Exposure 2
Figure 1D

Figure S7

Figure 2 Raw

Figure 2B

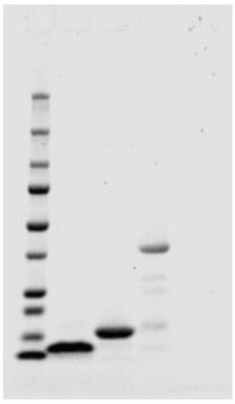

Figure 2C Exposure 1

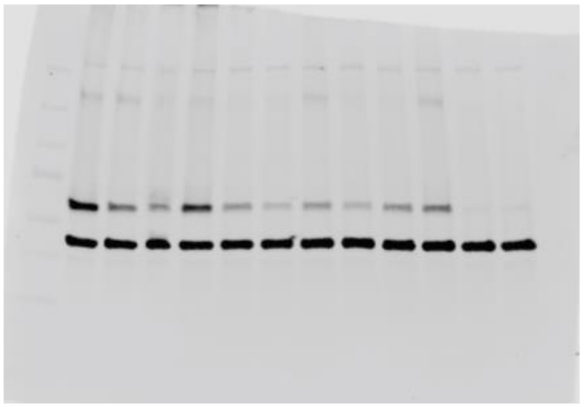

Figure 2C Exposure 2

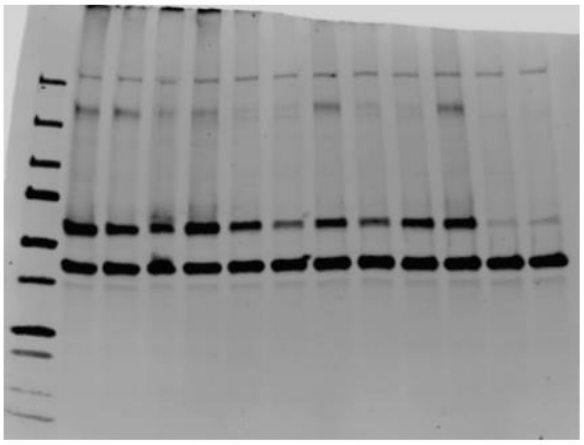

Figure 2D

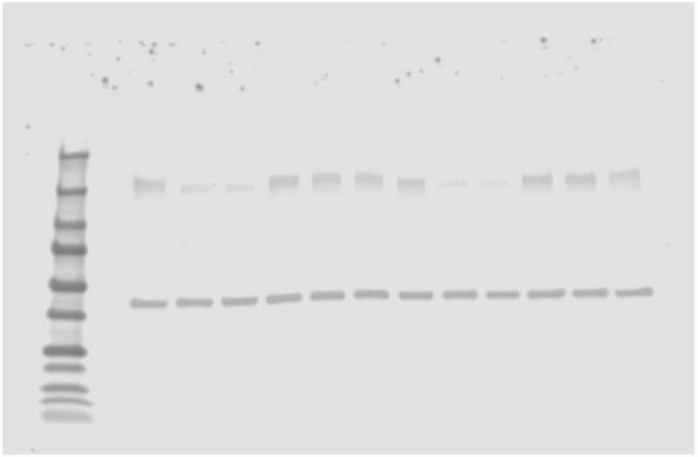

Figure S8

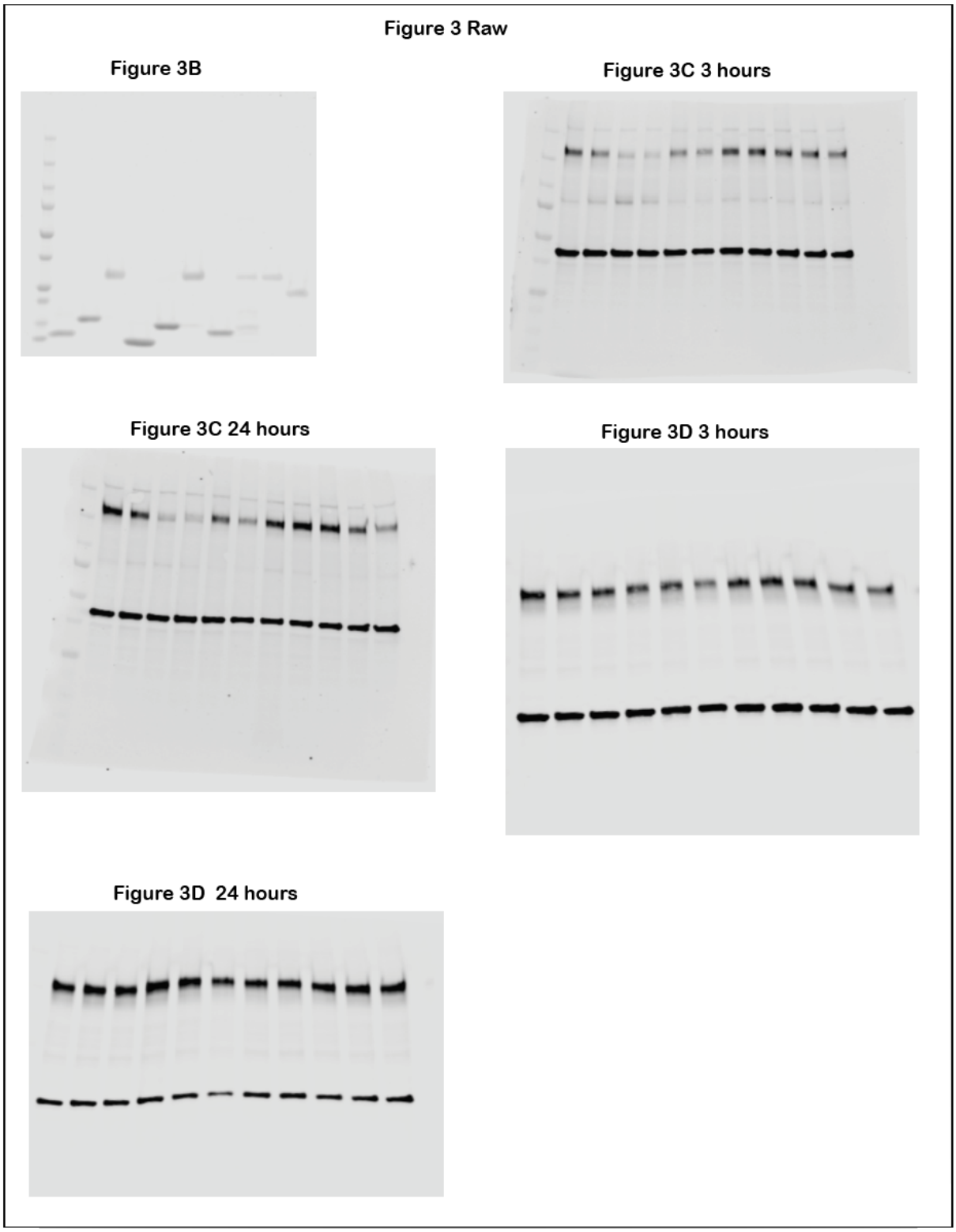
Figure 3 Raw
Figure 3B
Figure 3C 3 hours
Figure 3C 24 hours
Figure 3D 3 hours
Figure 3D 24 hours

Figure S9

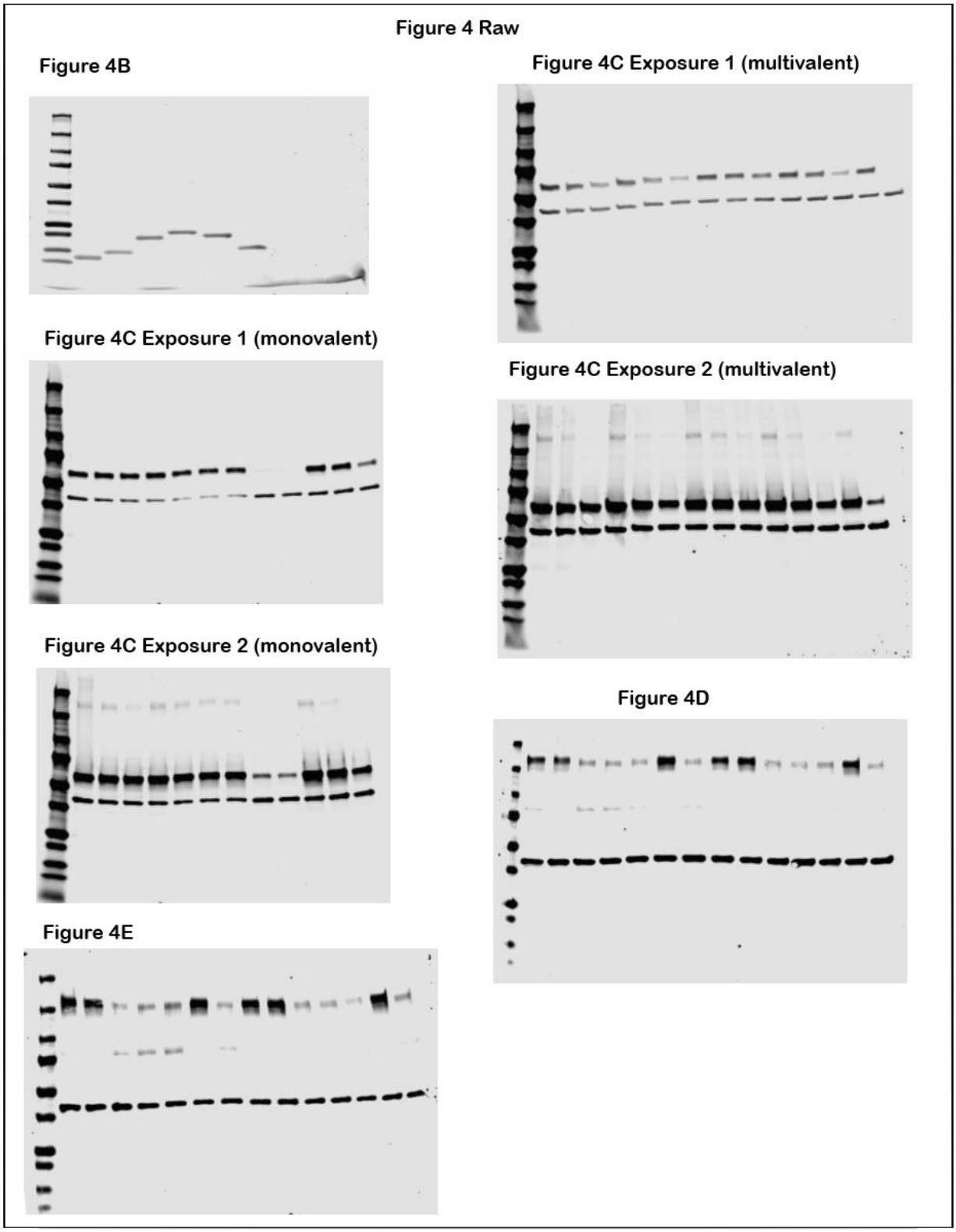
Figure 4 Raw
Figure 4B
Figure 4C Exposure 1 (multivalent)
Figure 4C Exposure 1 (monovalent)
Figure 4C Exposure 2 (multivalent)
Figure 4C Exposure 2 (monovalent)
Figure 4D
Figure 4E